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THE GAS BLADDER AND ITS RELATION TO THE INNER EAR IN *SARDINOPS CAERULEA* AND *ENGRAULIS MORDAX*

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THE GAS BLADDER AND ITS RELATION TO THE INNER EAR IN *SARDINOPS CAERULEA* AND *ENGRAULIS MORDAX*

By CHARLES P. O'CONNELL, *Fishery Research Biologist*

The California Cooperative Oceanic Fisheries Investigations [1] is primarily concerned with determining the causes of fluctuations in the abundance and availability of commercially important pelagic marine fishes. The extent to which a fish population is available to a fishery is a result of the interaction between the fish and the physical, chemical, and biological features of the environment. Changes in the environment result in changes in availability. Although many environmental features are being measured, very little is known about their effect on the behavior of fishes.

One approach to an understanding of availability is the investigation of behavior in the laboratory and in the field. Prior knowledge of the anatomy and physiology of the animal, particularly in regard to its sensory systems, is a prerequisite to such studies.

The Pacific sardine, *Sardinops caerulea*, and the northern anchovy, *Engraulis mordax*, are both commercially important species on the west coast of North America and are being studied by the California Cooperative Oceanic Fisheries Investigations. (These species will be referred to as sardine and anchovy in the text of this paper.) Since the behavior of both species will be studied in the near future, an anatomical study of each species has already been initiated.

The first organ system of these species to be examined was the gas bladder.[2] It became apparent, during the course of this work, that published descriptions of the gas bladder in other clupeoid fishes cannot, in all respects, be applied to the sardine and anchovy. For this reason, and also because the gas bladders of the sardine and anchovy exemplify certain marked anatomical contrasts that occur among clupeoids, the organ of each species is described in this paper.

The gas bladder in clupeoids is a retroperitoneal, tubular vesicle extending the whole length of the body cavity. It is connected to the alimentary canal by a pneumatic duct which arises from various positions along the dorsal wall of the cardiac stomach. In some species, the bladder opens to the exterior behind the anus; in others, it ends above the anus. In all clupeoid species, two capillary tubes, one on each side of the vertebral column, extend from the anterior end of the bladder into the cranium, where they expand into terminal vesicles encapsuled in bone. The two pairs of bony capsules are termed the prootic and pterotic bullae, in reference to skeletal elements involved. The anterior pair of bullae is closely associated with the inner ear. This relation, first described by Weber (1820) in *Clupea harengus*, involves the least understood, but most extensively investigated, feature of the bladder.

Apparently such gas-filled bony bullae are characteristic of all clupeoids, since Svetovidov (1950) was able to ascertain their presence in more than 50 species. As early as 1891, Ridewood described differences in the shape of these bullae, or capsules, in 6 species, and De Beaufort (1909) added to this information by describing such differences in more than 20 species, representing 10 genera. Despite differences in shape and proportion, the relation between these chambers and the ear is basically the same in all species. Tracy (1920 a) described the relation between the gas vesicles and the ear in *Pomolobus pseudoharengus*, with observations on a few other American species, and in another paper (1920 b) he gave a detailed

[1] A program sponsored by the Marine Research Committee and carried out cooperatively by the Scripps Institution of Oceanography of the University of California, the Bureau of Marine Fisheries of the California Department of Fish and Game, the South Pacific Fishery Investigations of the United States Fish and Wildlife Service, the Hopkins Marine Station of Stanford University, and the California Academy of Sciences.

[2] The author is in agreement with Woodland (1911) in regard to the terminology applied to this organ. Woodland states that "The terms swimbladder and airbladder are inappropriate, since fishes do not use the apparatus for swimming and it does not contain air." The term, "gas bladder," on the other hand, properly describes the organ without these implications.

description of each cranial bone involved. Wohlfahrt (1936), using *Clupea pilchardus*, has given by far the most detailed description of the whole mechanism.

I wish to express my gratitude to Dr. Rolf L. Bolin, Stanford University, for reviewing this paper, and for the many valuable suggestions he made. I would also like to thank George M. Mattson for his excellent drawings of the sardine and anchovy skulls.

RELATION BETWEEN THE GAS BLADDER AND EAR

GENERAL DESCRIPTION

In view of Wohlfahrt's work, only a general description of the relation between the ear and gas bladder in the sardine and anchovy will be presented here. Examination of sectioned cranii of the sardine and the anchovy showed the relation in these two species to be basically the same as it is in *Clupea pilchardus*. There are, of course, differences in the form of the cranial bones, but these probably do not modify the function of the ear–gas bladder mechanism. To study such differences in the sardine and the anchovy, skeletons of adult specimens were prepared according to the method described by Bolin (1935). Examination of articulated and disarticulated cranii was supplemented by dissections of formalin-preserved specimens.

Figure 1 shows, schematically, a dorsal view and lateral view of the ear–gas bladder relation on the left side of the cranium. The plane of the lateral view is indicated on the dorsal view; however, the dorsal view cannot be oriented in this way, because it shows structures at slightly different levels.

In most species the capillary extension of the bladder, immediately after it enters the exoccipital, expands slightly to form a small fusiform chamber in that bone. Anterior to this, the gas tubule bifurcates to produce a spheroid vesicle in the pterotic and a cylindrical vesicle in the prootic. The vesicles are enclosed in the hard, bony bullae already mentioned. The anterior, or prootic, bulla is divided into an upper and a lower chamber by a heavy, elastic, transverse membrane. The gas vesicle occupies the lower chamber, and in the wall of the upper chamber there is a transverse slit called the prootic fenestra. Through this sl the upper, perilymph-filled chamber is continuo with the large perilymphatic space under the flo of the recessus utriculus. The latter is strongl attached to the prootic bulla along the anteri lip of the fenestra. Between the posterior lip the fenestra and the floor of the recessus, which a slightly separated, there is a thin sheet of loo connective tissue. The sparse and widely sep rated fibers of this tissue do not interrupt the co tinuity between the perilymph in the bulla and i the space beneath the recessus.

The pterotic bulla is located within the loop the horizontal semicircular canal, which is n shown in the figure. The gas vesicle is completel enclosed by the bulla, and closely applied to i inner surface.

The nervus acousticus (VIII) innervates seve sensory areas of the membranous labyrinth, as i most fishes. So far as is known, the clupeoids a the only group in which the ramulus utriculu (one of the seven branches) is divided into thre parts. These branches extend laterally under th floor of the recessus utriculus: the anterior branc along the anterior lip of the prootic fenestra, th medial branch approximately above its posterio lip, and the posterior branch over the larg perilymphatic space. In figure 1 these nerv branches are indicated though not labeled. Eac branch innervates an overlying macula in th recessus. The lapillus is seated on the media and posterior maculae and attached to the anterio macula by a horizontal membrane that is trian gular in cross section.

Wohlfahrt found the axis cylinders of th ramulus utriculus anterior in *C. pilchardus* to b smaller in diameter than any others in the ramu anterior VIII, but the same as those in the ramu posterior VIII. This condition has also bee observed in the sardine and anchovy.

The membranous labyrinth of clupeoids i unique in being differentially thickened, and i having definite perilymphatic ducts runnin through some of the thickened portions. One o the most noticeable thickenings of the wall under lies the macula utriculus media, and the thinnes region is between it and the macula utriculus an terior, or directly above the prootic fenestra. Th perilymphatic systems surrounding the two mem branous labyrinths are, furthermore, continuou

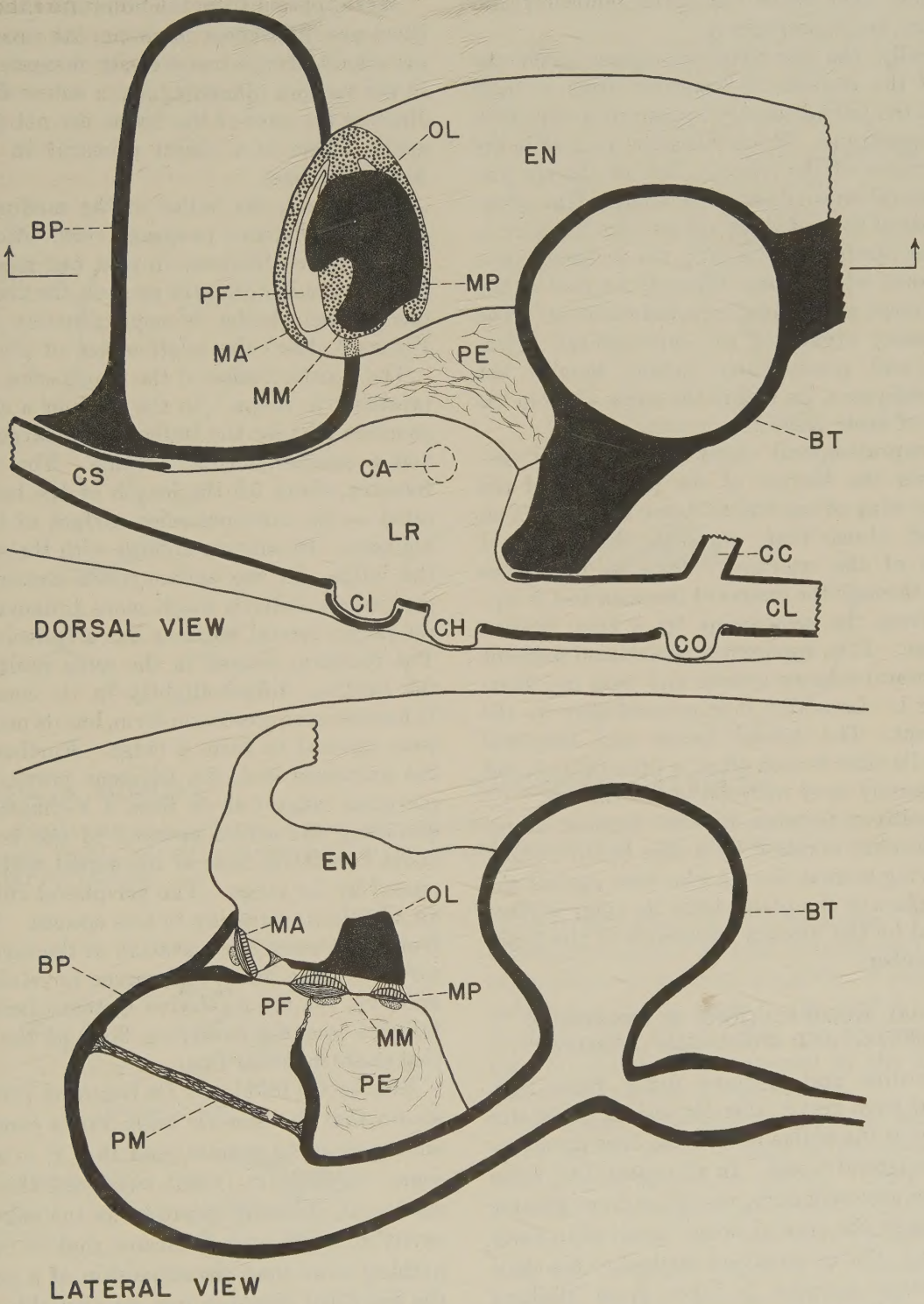


FIGURE 1.—Schematic drawing of relation between membranous labyrinth and gas vesicles on the left side of the clupeoid cranium. The plane of the lateral view is marked on the dorsal view. The solid black areas represent bone. *BP*, prootic bulla; *BT*, pterotic bulla; *CA*, accessory temporal sensory canal; *CC*, occipital sensory canal; *CH*, preopercular sensory canal; *CI*, infraorbital sensory canal; *CL*, lateral sensory canal; *CO*, opercular sensory canal; *CS*, supraorbital sensory canal; *EN*, endolymphatic space; *LR*, lateral recess; *MA*, macula utriculus anterior; *MM*, macula utriculus media; *MP*, macula utriculus posterior; *OL*, otolith (lapillus); *PE*, perilymphatic space; *PF*, prootic fenestra; *PM*, prootic membrane.

with each other below the brain anteriorly and above the brain posteriorly.

Laterally, the perilymphatic space under the floor of the recessus is separated from a large sinus of the lateral-sensory system by a very thin elastic membrane. From this sinus, located in the lateral recess of the cranium, five of the six primary lateral-sensory canals emanate. The proximal ends of four of these are obvious in figure 1, while the opening of the fifth, the accessory temporal canal, which passes through the roof of the lateral recess, is indicated by a dashed circle. The first sensory organs of the supraorbital, infraorbital, and preopercular canals, though not shown in figure 1, lie within the recess close to the mouths of their respective canals.

The supratemporal canal system, which extends over the surface of the parietal and the posterior wing of the frontal bone, arises from an expanded sinus that occupies the temporal foramen of the cranium. Mesially, this sinus projects through the temporal foramen and is separated from the ectomeninx by a thin hyaline membrane. It is, apparently, an isolated segment of the lateral-sensory system and does not show in figure 1. Laterally, it is covered only by the integument. The lateral recess and temporal foramen lie close to each other, a little behind, and approximately level with the top of the eye.

The auditory fenestra, an oval window lateral to the sacculus, is closed by a thin hyaline plate. In the living animal, the sacculus rests against the inner surface of the plate, while its outer surface is covered by the mucous membrane of the branchial chamber.

CRANIAL MODIFICATIONS IN *SARDINOPS CAERULEA* AND *ENGRAULIS MORDAX*

The sardine and anchovy differ from each other, and from many other clupeoids, in the size and shape of the bullae and in the bone construction of the lateral recess. In all probability, these differences are secondary, resulting from greater differences in the cranial bones involved in their formation. The terminology applied to the skull of *Sardinops caerulea* is taken from Phillips (1942), and since there is no published osteological description of *Engraulis mordax*, the same terminology has been followed as far as it was applicable to the anchovy.

It is apparent from the literature that, althoug there are differences in shape, the prootic bull are almost always transversely elongate, and me in the median plane to form a subcerebral ridg Because the axes of the bullae are not quite hor zontal, there is a slight dihedral in the ridg formed by them.

In figure 2, the bullae of the sardine and a chovy are sketched in dorsal view. Most notic able is their difference in size, but since the a chovy is smaller than the sardine, the proportion size of their bullae is approximately the sam Figure 3 shows the relative size of the cranii.

The prootic bullae of the two species differ ap preciably in shape. In the sardine, a slight con striction divides the bulla into a larger, latera and a smaller, medial portion. The crescenti fenestra, about 0.3 the length of the bulla, is lo cated on the dorsoposterior surface of the large segment. Its margin is flush with the surface o the bulla. In the anchovy, the constriction i the prootic bulla is much more pronounced, an the larger lateral segment has a spherical shape The fenestra, located in the same position as i the sardine, differs slightly in its construction It has the same crescentic form, but its margin pro jects upward to form a ridge. Furthermore, a the narrowed end, the adjacent portions of the marginal ridge join to form a V-shaped groove, occluding the actual opening of the fenestra to about two-thirds that of the actual space encompassed by the ridge. The peripheral ridge is not an additional structure in this species. It results from differences in excavation of the surrounding surface of the bulla, and serves to retain, in the anchovy, the same relative distance between the fenestra and the overlying floor of the recessus that exists in the sardine.

Ridewood (1891) and De Beaufort (1909) have shown that the pterotic bulla varies considerably among clupeoid species, and that it is absent in some. Wohlfahrt (1936) suggested that its formation in the same position as the subtemporal cavity of cyprinoids indicates that it represents nothing more than the expansion of a portion of the gas-filled chamber into an available space in the cranium.

The pterotic bulla is well developed in both the sardine and anchovy. In each, it is separated by a slight constriction into a smaller lower chamber

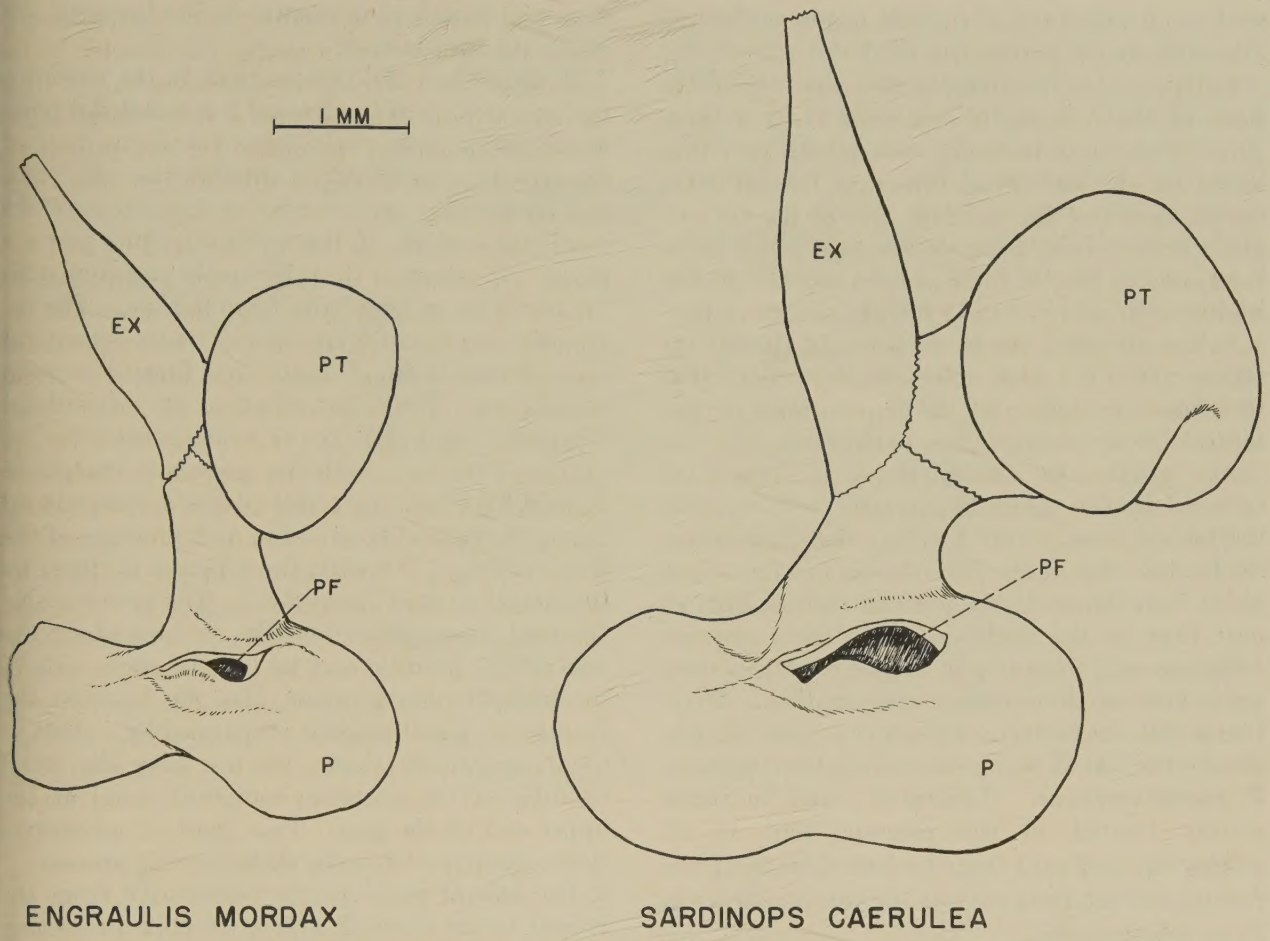


FIGURE 2.—Dorsal view of bony bullae removed from the left side of the cranii of *Sardinops caerulea*, 220 mm. standard length (right), and *Engraulis mordax*, 150 mm. stan dard length (left). *EX*, exoccipital; *P*, prootic; *PF*, prootic fenestra; *PT*, pterotic.

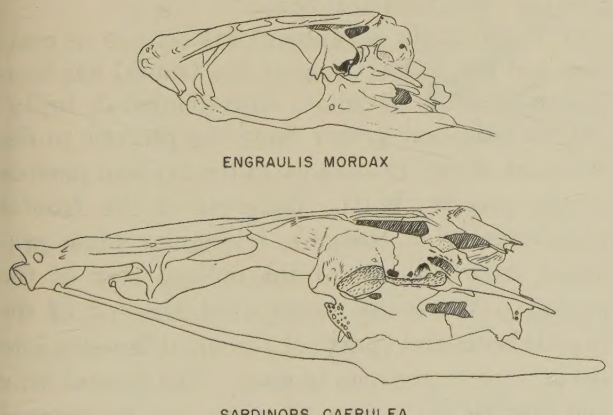


FIGURE 3.—View of left side of cranii of *Sardinops caerulea*, 264 mm. standard length (below), and *Engraulis mordax*, 147 mm. standard length (above), to show relative difference in size between adults.

and a larger, spheroid upper chamber. The upper chamber projects somewhat anteromesially, but in the sardine the anterior projection is more noticeable, while in the anchovy the mesial projection is more emphasized. The constriction between the two chambers is traversed by the horizontal semicircular canal in both species.

The extent to which the pterotic bulla is exposed on the outer surface of the cranium also differs from species to species. Tracy (1920 b) found that the capsule protruded slightly into the pre-epiotic fossa in *Pomolobus pseudoharengus*, was only slightly visible in *P. mediocris*, was covered except for a microscopic area in *Brevoortia tyrannis*, and was completely covered in *Alosa sapidissima*. These differences result from the extent to which the bulla is covered by the expansion of the pterotic bone. In both

sardine and anchovy, the whole upper surface of the bulla projects into the temporal groove between the temporal foramen and the pre-epiotic fossa (fig. 4). It is entirely covered by muscle, which in turn is partially covered by the thin, triradiate supratemporal bone. In the anchovy, the supratemporal completely overlies the surface of the bulla, while in the sardine part of the bulla is exposed in the temporal groove anterior to the leading edge of the supratemporal.

In the anchovy, the bones forming the lateral recess are thin and the lateral-sensory-canal foramina are relatively large, whereas in the sardine the bones are thick and the foramina relatively small. In the anchovy, the recess is bounded by the sphenotic, prootic, pterotic, and the lateral wing of the frontal. In the sardine, the lateral wing of the frontal does not form part of the wall; however, the pterotic forms a greater part than in the anchovy. *Pomolobus pseudoharengus* and *Clupea pilchardus* show the same differences as the anchovy and sardine. Tracy found that the posterior face of the sphenotic was deeply excavated to accommodate the recess in *P. pseudoharengus*. Wohlfahrt found the recess mainly located in the pterotic bone in *C. pilchardus*, and said that the lateral wing of the frontal did not form part of the recess wall, as in *P. pseudoharengus*.

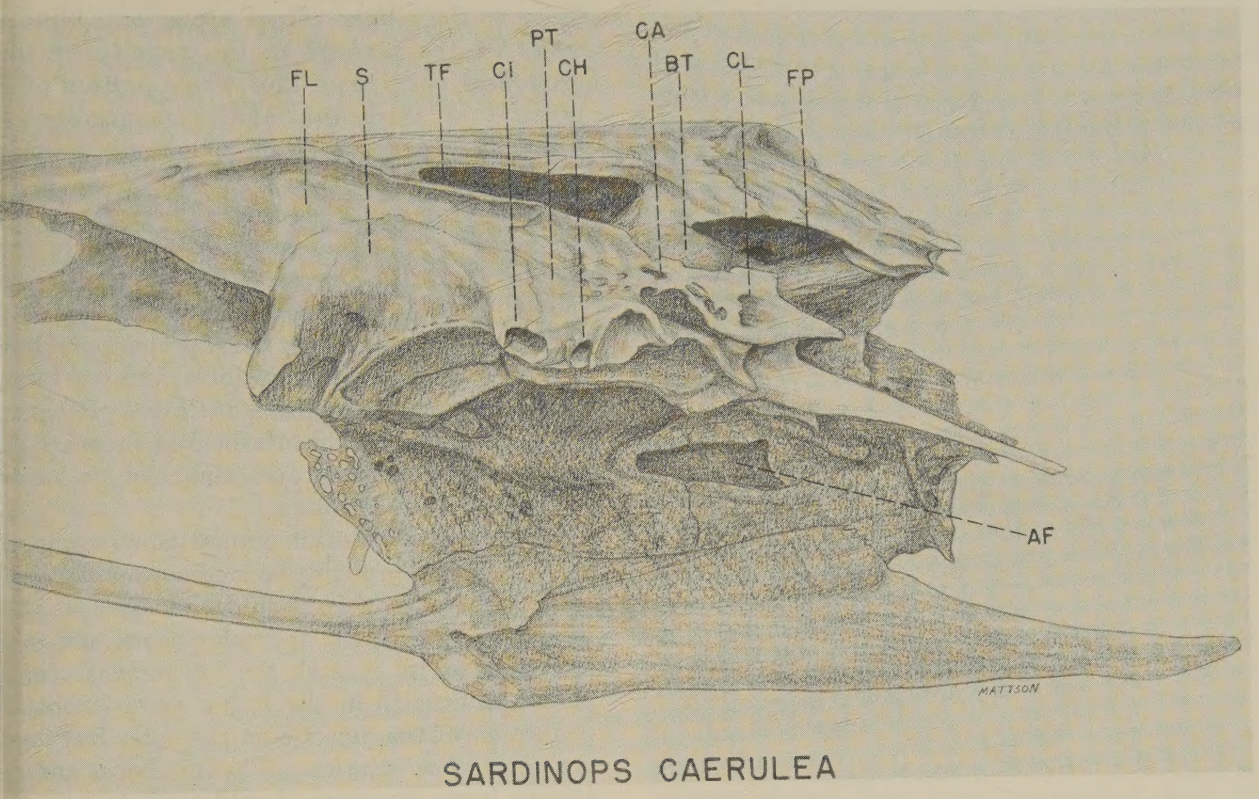
Except for the mesial communication with the auditory recess, which is interrupted by a thin elastic membrane, the only openings in the lateral recess are those of the lateral-sensory canals. The supraorbital canal runs anteromesially in the bony tube formed by the lateral wing of the frontal. The lateral canal passes posteriorly through the pterotic bone from the lateral recess. As it emerges, the opercular and exoccipital canals branch from it, and then it continues downward external to the pectoral girdle. The supraorbital and lateral canals give the impression of being one continuous tube, expanded as a sinus in the region of the lateral recess. The infraorbital, preopercular, and accessory-temporal canals open from the sinus at approximately right angles to the axis of this tube. The first two canals open laterally, one behind the other, while the third opens dorsally. Although identical lateral-sensory-canal foramina occur in both species, their sizes and locations in respect to the bones of the recess are not the same.

In figure 4, it can be seen that, in the anchovy, the lateral wing of the frontal is a broad, flat plate which forms most of the lateral wall and roof of the recess. Anteriorly, it overlies the sphenotic, and its curving ventroposterior margin parallels contiguous edges of the sphenotic and pterotic bones. It recurves dorsally to its junction with the temporal ridge of the frontal bone. The infraorbital, preopercular, and accessory-temporal canals emanate from the slit thus formed between these bones. The anterior half of the infraorbital foramen is formed by the excavated posterior face of the sphenotic, while its posterior margin is formed by a bony stay that projects obliquely up from the pterotic bone to the undersurface of the frontal wing. Dorsally, the foramen is closed by the lateral wing of the frontal. The preopercular foramen, incomplete dorsally, is formed by the wall of the pterotic and its oblique stay. Above the preopercular foramen, the gap between the frontal wing and pterotic is spanned by a sheet of tough connective tissue, leaving only the small opening of the accessory-temporal canal at the upper end of the gap. This sheet of connective tissue disappeared in the skeletonizing process.

The lateral canal passes posteriorly from the lateral recess through a loop of bone projecting from the pterotic. The lateral segment is a narrow stay, but the mesial segment is produced posteriorly as a broad wall with a flared margin. At this point, the opercular canal branches ventrally and the occipital canal branches dorsally to pass into the supratemporal bone.

In figure 5, the supratemporal bone is in position and the lateral wing of the frontal has been removed. The triradiate supratemporal, bridging the temporal groove from the pterotic to the occipital, is well in advance of the exposed portion of the pterotic bulla. Removal of the frontal wing exposes an elongate, triangular plate produced from the end of the oblique stay of the pterotic. When this is removed, the end of the prootic bulla and (part of) the small fenestra into the auditory recess can be seen. The frontal wing and pterotic projection, of course, can be removed together.

In the anchovy, the greatest lateral projection of the cranium is the wing of the pterotic that re-



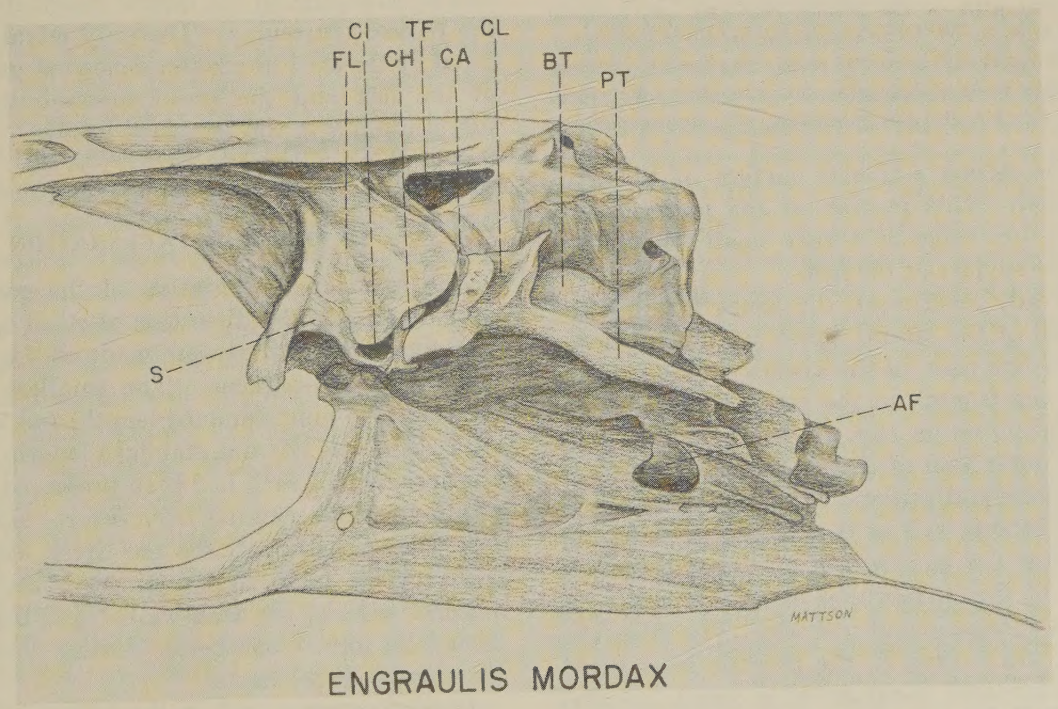


FIGURE 4.—View of left side of postorbital region of cranii of *Sardinops caerulea* (264 mm. standard length) and *Engraulis mordax* (147 mm. standard length). *AF*, auditory fenestra; *BT*, pterotic bulla; *CA*, accessory temporal sensory canal; *CH*, preopercular sensory canal; *CI*, infraorbital sensory canal; *CL*, lateral sensory canal; *FL*, lateral wing of frontal; *FP*, pre-epiotic fossa; *PT*, pterotic; *S*, sphenotic; *TF*, temporal foramen.

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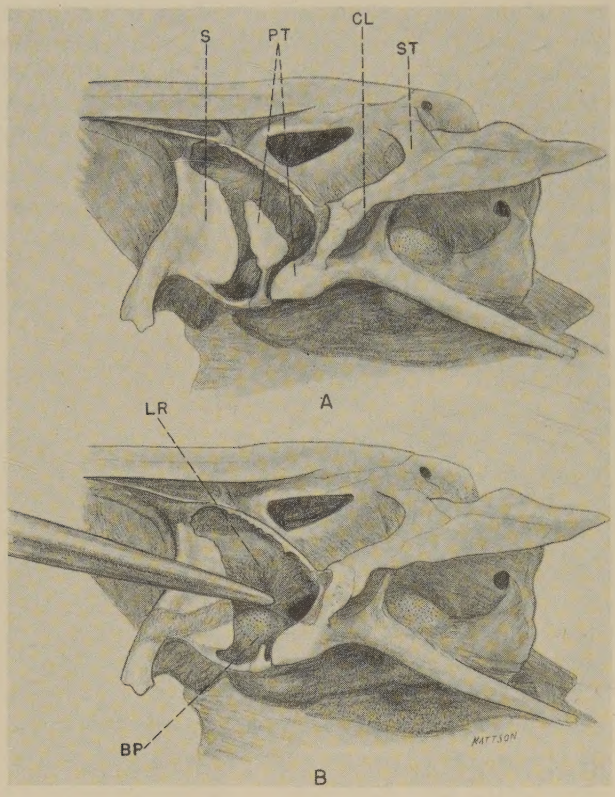

FIGURE 5.—Lateral view of cranium of *Engraulis mordax*. A. Lateral wing of the frontal removed. B. Pterotic extension removed. The dissecting needle points to the fenestra between the lateral recess and auditory recess. *BP*, prootic bulla; *CL*, lateral sensory canal; *LR*, lateral recess; *PT*, pterotic; *S*, sphenotic; *ST*, supratemporal.

ceives the second articular surface of the hyomandibular. This portion of the pterotic, furthermore, protrudes as a very small but distinct nob on the surface of the fish.

As already mentioned, the bones forming the outer wall of the lateral recess are much thicker in the sardine than in the anchovy. The lateral wing of the frontal, in the sardine, does not extend over the recess (fig. 4). It forms no more than the outer wall of the supraorbital tube, and the sphenotic and pterotic bones meet below it to form the lateral wall of the recess. A short distance below the junction of all three bones the foramen of the infraorbital canal intervenes between the sphenotic and pterotic. The other two foramina are located in the pterotic bone itself. The preopercular foramen is a small, circular opening a little distance behind the infraorbital foramen, and the accessory-temporal foramen is an even smaller opening dorsal and slightly posterior to the latter. There are several blind recesses of the pterotic in the vicinity of these prominent openings. This whole portion of the pterotic is thickened, and, consequently, the foramina are in the form of tubular passages through the bone.

The lateral canal passes from the recess through a long bony tube that emerges posteriorly from the surface of the pterotic. The terminus of this tube is expanded as a flat, triangular surface to accommodate a portion of the supratemporal bone.

Wohlfahrt described one dorsal and two lateral openings in the lateral recess of *Clupea pilchardus*. These, of course, are the infraorbital, preopercular, and accessory-temporal foramina, and are located exactly as in the sardine.

Owing to differences in cranial construction, the anchovy would probably be more amenable to surgical techniques than would the sardine. The lateral-sensory canals, opercular bones, and other structures in or beneath the integument are not visible externally in the living animal, but the projection of the pterotic on the outer body wall is a point of orientation. The thin bones and relatively large foramina should permit easier access to structures in the lateral recess and around the recessus utriculus. The only advantages offered by the sardine are the somewhat greater size of the fish and the easier accessibility of the pterotic bulla in the temporal groove.

THE GAS BLADDER

METHODS OF PREPARATION

The form and orientation of the gas bladder were determined by dissection of adult specimens. Eight such dissections were made of the anchovy, and 14 of the sardine. The smallest anchovy used was 145 mm. standard length, and the smallest sardine was 170 mm. standard length. All but one specimen of each had been preserved in formalin for periods ranging from several months to a few years. Although the one fresh example of each species facilitated distinction between the visceral organs, the thin-walled gas bladder retained its form much better during dissection in the preserved specimens.

Dissections were made in the following manner: The left wall of the body cavity was removed from the pectoral girdle to a point just short of the anus. The opercle and branchial arches on the left side

ere also removed. The ribs and enough of the teral muscle tissue were removed to expose the ertebral column. After excision of the left go- nd, fat bodies, and the intestine, the stomach was oved down slightly to permit a better view of the as bladder. Certain features of the bladder and omach were then oriented in respect to the ver- bral column, the dorsal and anal fins, and the nus. After this the gas bladder was removed nd placed in a dish of 10-percent formalin for urther examination and measurement. Bladders om preserved specimens were punctured in a w places so that liquid could enter the lumen and xpand them fully.

Serial sections were made of some of the speci- ens, fixed in 10-percent formalin, to study the istology of the bladder wall. All preparations ere dehydrated in normal butyl alcohol and em- edded in 54° to 56° rubber paraffin *in toto*. The locks were then cut into four or five portions, ach of which was sectioned separately. Sections ere cut at 10 or 12 microns, and attached to slides ith Haupt's gelatin adhesive. A 0.5-percent queous solution of potassium dichromate was used o expand the sections on the slides.

All preparations were stained with a modifica- on of Van Gieson's trichrome stain. Mayer's emalum was used in place of Weigert's iron ematoxylin as a nuclear stain. Curtis' substitute Leach 1946) was used in place of the acid uchsin–picric acid counterstain employed by Van ieson. It consists of 2-percent aqueous ponceau , 5 cc.; saturated aqueous solution of picric acid, 5 cc.; 2-percent acetic acid, 2 cc.

Mayer's hemalum, although it does not resist estaining by the counterstain as well as Wei- ert's hematoxylin, was employed because it is elatively simple to apply and retains its strength or a much longer time. The advantages of Cur- s' modified counterstain are that it stains eticular as well as collagen fibers and does not ade. As with Van Gieson's stain, Curtis' modi- cation stains nuclei blue, muscle tissue yellow, nd connective tissue red and various shades of rown. In practice it was found that the muscle ssue sometimes had a spotty overtone of orange, ut this condition was almost entirely overcome by oubling the proportion of picric acid given by each.

The preparation represented in figures 12 and 13 differs from the others in that it was dissected from a freshly killed specimen and fixed in Zenker's fluid, then decalcified in a 2-percent solution of nitric acid in 70-percent ethyl alcohol.

ORIENTATION AND FORM

In figure 6, the gas bladder and part of the digestive tract of the sardine and the anchovy are outlined. The standard lengths of the two specimens are equilibrated so that differences are proportionate. The body cavity, and consequently the gas bladder, is relatively shorter in the anchovy. In the sardine, the pneumatic duct originates from the posterior end of the blindsac, or fundus of the stomach, the bladder has only one chamber and has a postanal opening to the exterior. In the anchovy, the pneumatic duct originates from the dorsal wall of the cardiac stomach, the bladder is composed of two chambers and lacks the postanal opening. Although these two species differ in the foregoing characters, the two combinations do not constitute alternative patterns within the clupeoids. De Beaufort (1909) has already shown that these anatomical features occur in various combinations.

The anterior capillary extension of the bladder also differs in the two species, but the bifurcated anterior portion of the single median tube in the anchovy is homologous with the pair of capillaries arising directly from the anterior end of the bladder in the sardine. The proximal end of the paired capillaries is located under the fourth vertebra in each species, and in each it embraces the ascending dorsal aorta. Thus, it is possible to compare the bladders proportionately by taking their lengths from bifurcation to posterior tip as 100 percent.

In figure 7, there are four drawings of bladders from the sardine and three of bladders from the anchovy. Numbers 1 and 5 depict the most typical form for each species; the others illustrate variations. Comparing the typical forms, the most striking difference is in the location of the greatest expansion; in the sardine it occurs in the anterior half of the bladder, in advance of the insertion of the pneumatic duct, while in the anchovy it occurs in the posterior half, behind the insertion of the pneumatic duct. The portion behind the duct in the sardine attenuates as it approaches the postanal opening. At the posterior end of the bladder

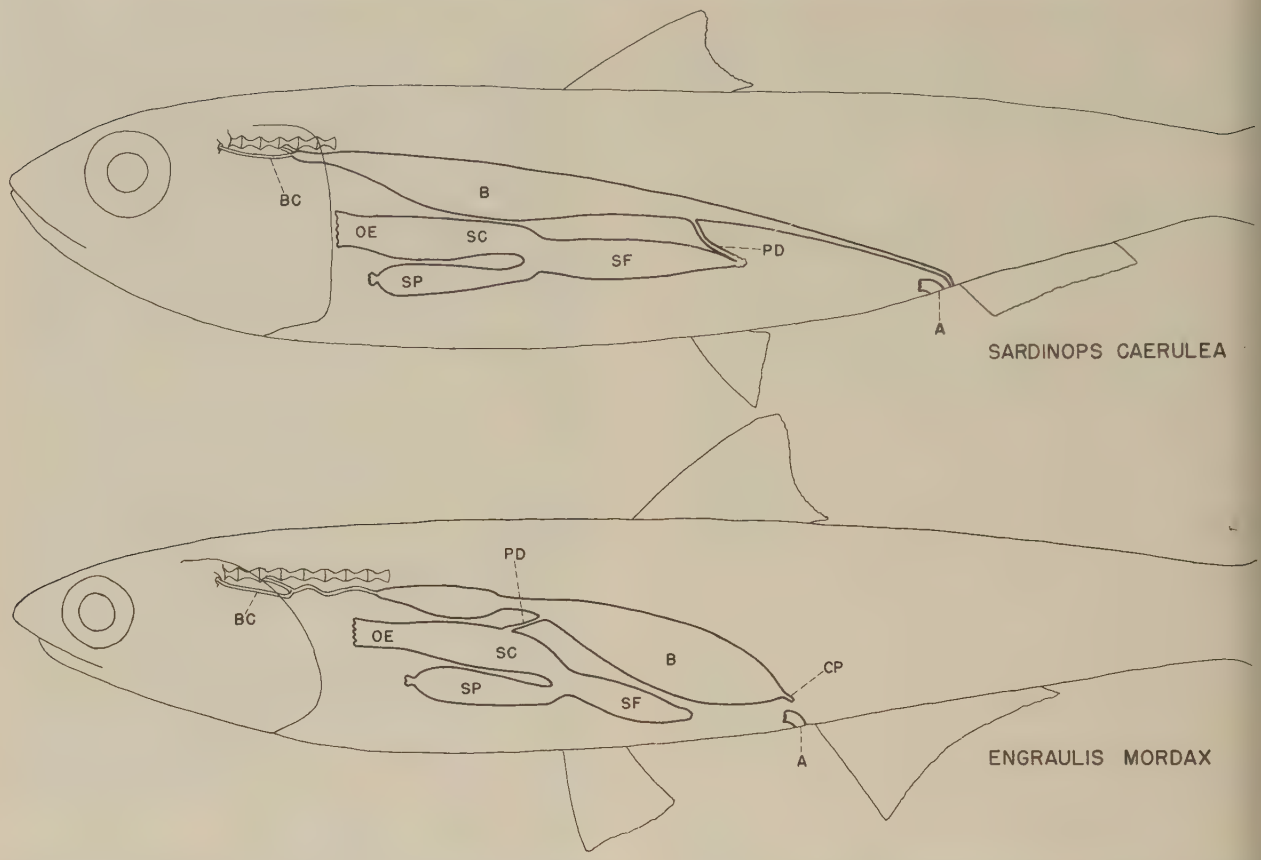

FIGURE 6.—Form and orientation of gas bladder and digestive tract in *Sardinops caerulea* (200 mm. standard length) and *Engraulis mordax* (145 mm. standard length). The standard lengths are equilibrated so that comparisons are proportionate. *A*, anus; *B*, gas bladder; *BC*, precoelomic capillary of gas bladder; *CP*, posterior caecum; *OE*, esophagus; *PD*, pneumatic duct; *SC*, cardiac stomach; *SF*, fundus of stomach; *SP*, pyloric stomach.

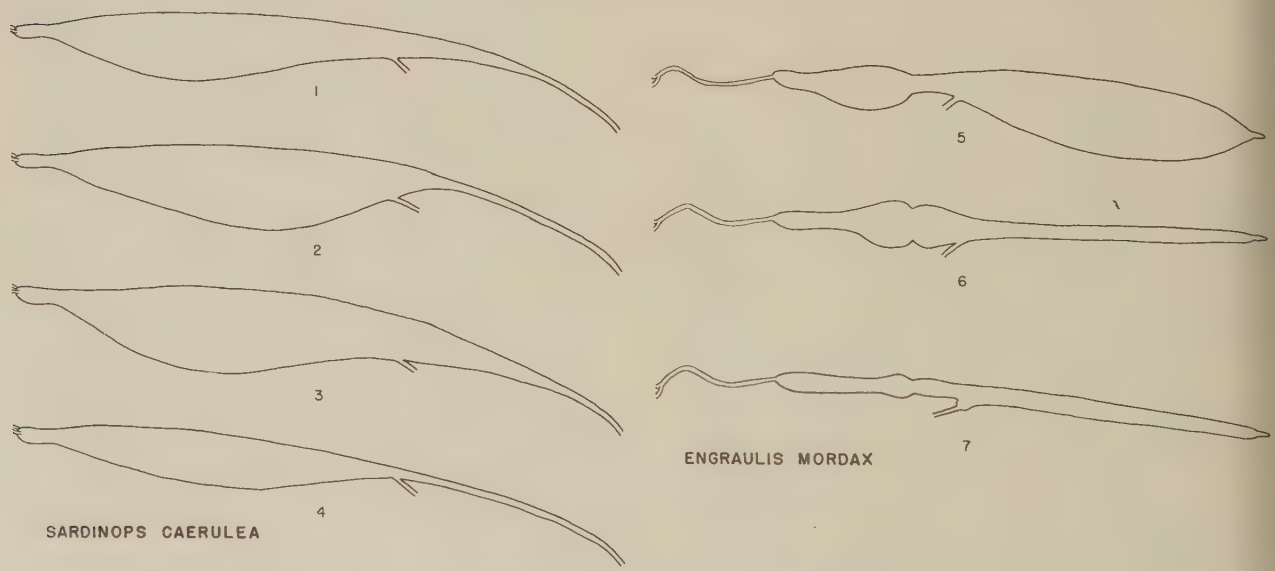

FIGURE 7.—Proportionate comparison of forms of the gas bladder in *Sardinops caerulea* and *Engraulis mordax*. Numbers 1 and 5 represent the typical form for each species, respectively; the others represent variations noted in dissection of preserved specimens.

the anchovy, there is a short blind caecum. In the sardine, the only anterior differentiation is a very short, constricted portion of the bladder intervening between the expanded chamber and the bifurcation. In the anchovy, on the other hand, the portion anterior to the duct is composed of three distinct segments: a relatively short, constricted portion, an expanded but small anterior chamber, and a relatively long median-capillary tube that extends to the bifurcation. This median capillary, as well as the paired ones extending forward from it, is sheathed in cartilage, as are the paired capillaries in the sardine.

Variation in the shape of the bladder among the specimens examined was most marked in the anchovy, in which at times the large posterior chamber is contracted to tubular proportions, or the middle constricted segment is expanded to five or six times its usual diameter. No matter what the condition, the position of the transverse septum (see p. 519) is marked by a noticeable constriction, and the distance between the septum and the insertion of the pneumatic duct is proportionately the same.

Variation of form in the sardine is most noticeable in the expanded portion forward of the pneumatic duct, although I have not found it contracted to the degree that the posterior chamber may be in the anchovy. The portion behind the duct varies from a tapering, or triangular, form to a contracted, tubular one. When the anterior half is well expanded, the posterior half may be either tubular or tapering (numbers 2 and 3), but whenever the anterior half is contracted to any noticeable degree, the posterior half appears always to be tubular (number 4).

It is impossible to state here that these variations in the form of the bladder exemplify natural physiological conditions; however, they do illustrate the capacity of the bladder to expand and contract differentially. They are not attended by any evidence of rupture or folding.

THE PNEUMATIC DUCT

Some earlier workers have described the pneumatic duct of certain clupeoids as having a small lumen running tortuously through the tissue of the duct. Later investigators described a bulbous structure at the lower end of the duct in cases where the duct arises from the end of the blindsac of the stomach. Maier and Scheuring (1923), however, were the first to give a detailed description of this "bulb" in the herring, *Clupea harengus*. They described the duct as arising ventrally from the posterior end of the blindsac, the separation being effected by a horizontal partition arising from laterally opposed, longitudinal folds of the stomach mucosa. In the duct itself, they counted 15 to 17 overlapping septa. There is no similarly detailed description of the pneumatic duct in cases where it arises from other parts of the cardiac stomach, such as it does in the anchovy.

Sardinops caerulea

Throughout its length, the pneumatic duct of the sardine is approximately 0.6 mm. in diameter and the wall is between 35 and 60 microns thick in a 200-mm. specimen. When viewed in sagittal section, it can be seen that the lower end of the duct is divided into a series of pockets by incomplete transverse septa, radially arranged in conformity with the sharp curvature of the duct at this point (fig. 8). These septa are attached only to the lateral and lower, or posterior, walls of the tube, forming a series of blind pockets facing forward toward the stomach. The continuous passage between the successive pockets follows the lesser curvature of the bend closely, and is slightly irregular, so that the openings through the septa are not perfectly alined. This irregularity varies considerably among individuals, and probably changes with the activity of the duct. Where the duct begins to straighten, the openings through the septa gradually assume a more central position. In one specimen, 12 septa were counted in the lower half of the duct. There are no septa in the upper half of the duct, and the difference between the two parts is apparent to the naked eye in some of the formalin-preserved specimens.

The upper and lower halves of the pneumatic duct differ in histological detail as well as in gross morphology. The epithelium in the lower half of the duct is a simple layer of tall, columnar cells, which closely resemble but are not the same as those of the stomach. The cells in the stomach have fusiform basal nuclei, while those in the duct have round basal nuclei. The cytoplasm also stains differently. The epithelium of the duct is elevated into longitudinal folds which extend over the septa to give the inner surface of the duct a reticulate appearance. Although the epi-

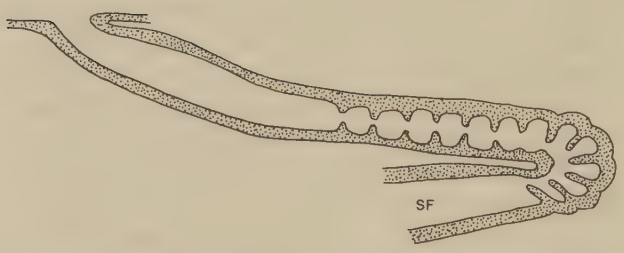

FIGURE 8.—Diagrammatic sketch of the pneumatic duct of *Sardinops caerulea*. *BL*, lumen of gas bladder; *SF*, fundus of stomach.

thelium and some of the folds are continuous between stomach and duct, the character of the cells changes abruptly at the first septal opening.

The submucosa in the stomach is a thick layer of dense connective tissue into which the gastric glands invaginate. It ends abruptly at the first septal opening, and apparently has no counterpart in the pneumatic duct. A thin layer of less-dense connective tissue, undoubtedly a part of the mucosa, is interposed between the epithelium and submucosa of the stomach, and is continuous with a similar layer in the pneumatic duct. The muscularis in both structures is composed of an inner layer of transverse smooth-muscle cells and a thinner, outer layer of longitudinal cells. Though the muscle coats of both structures have the same alinement, there is an oblique line of demarcation between them. The fibers are smaller in the duct than in the stomach, and stain a slightly different color. Furthermore, the outer, longitudinal layer is thinner in the duct.

From the arrangement of the tissue layers at the transition between the stomach and the duct, it may be tentatively concluded that the pneumatic duct is primarily an outgrowth of the mucosa of the digestive tract; and that the submucosa and muscularis of the stomach either end at the junction of the stomach and duct, or continue in the duct as incipient tissue layers, while the muscularis mucosa becomes prominent in the duct.

Although there are no transverse septa in the upper half of the pneumatic duct, a few of the longitudinal epithelial folds extend its entire length. The epithelial cells become cuboidal in the upper half of the duct, and the transverse muscle layer becomes much thinner, while the longitudinal layer is composed of more or less isolated loose bundles running longitudinally and obliquely in a thick layer of dense connective tissue. This connective tissue is continuous with that noted in the lower part of the duct. A section of this portion of the wall is shown in figure 10 at a magnification of × 680. Maier and Scheuring (1923) found a few very large, isolated fibers in the duct wall of *Clupea harengus*. Nothing fitting this description was found in the few specimens of the sardine examined, but it appears that the isolated longitudinal fibers were somewhat larger than the transverse fibers.

The lumen of the duct opens broadly into the bladder, and here the transverse muscle layer gradually becomes thickened. Aside from this, there is no specialized sphincter mechanism. Also at this point the longitudinal muscle fibers appear to invade the transverse muscle layer. The two types of fibers are intermingled in the adjacent region of the bladder wall, but, as described on page 519, one becomes dominant anteriorly and the other posteriorly.

Engraulis mordax

Externally, there is no evidence of a bulb in the pneumatic duct of the anchovy (fig. 9). Dissection and histological examination, however, show the duct to be septate over most of its length. For a short distance at each end, the epithelium forms high, longitudinal folds which traverse the septa, giving the inner surface of the duct a slightly reticulate appearance. The folds at each end of the duct occupy a considerable portion of the lumen. As it penetrates the dorsal wall of the stomach, the duct bends ventrally, and the septa are obliquely alined to the axis of the lumen. The openings through the septa are along the ventral wall, or lesser curvature, of the duct. Thus, although they arise from the opposite wall of the duct, the first few septa form blind pockets facing the stomach, as in the sardine. The diameter of the lumen is a little greater in the region of the bend

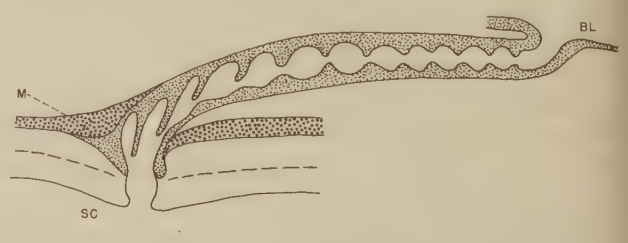

FIGURE 9.—Diagrammatic sketch of the pneumatic duct of *Engraulis mordax*. *BL*, lumen of gas bladder; *M*, striated muscle; *SC*, cardiac stomach.

an in the rest of the duct, where it is 0.8 mm., or ghtly less, in a 145-mm. specimen.

The epithelial cells are cuboidal throughout the ngth of the duct. At the lower end, there is a arp line of demarcation between epithelial cells the stomach and of the duct, and at the upper d the cells change gradually to the squamous ndition as the epithelium passes into the bladder.

The muscularis of the duct is composed of an ner layer of transverse smooth-muscle fibers and very thin, outer layer of longitudinal fibers. At e lower end of the duct, adjacent to the region f longitudinal folds, the muscularis forms a ightly thickened ring. It was not possible to etermine whether longitudinal as well as trans-rse fibers occurred here. This ring lies beneath e striated muscle coat and on a level with the bmucosa of the stomach. The arrangement of e tissues at this junction again suggests, as in e sardine, that the pneumatic duct is primarily erived from the mucosa of the digestive tract. he transverse muscle layer of the duct wall pro-cts into all the septa, and at the junction of the uct with the bladder it is considerably thickened.

unctions of the pneumatic duct

It is clear that the pneumatic ducts of the sar-ine and the anchovy are comparable in structure, nd it is highly probable that the duct performs e same functions in both species. It may act as valve or trap to permit gas but not food to pass owards the bladder. It may pump gas from the igestive tract to the bladder, or it may provide a assage for the escape of gas from the bladder. t could perform all three functions, which are not utually exclusive.

Maier and Scheuring (1923) interpreted the tructure of the "pneumatic bulb" in *Clupea harengus* as that of a valve which prohibits the en-rance of food particles (and parasites) into the neumatic duct. They hypothesized that the food ass, if compressed into the posterior part of the lindsac, would close the free edge of the first eptum against the stomach wall opposite the sep-um; and that any particles passing the first sep-um would in the same way be stopped by suc-eeding septa. They did not consider the manner n which such particles would be eliminated from he duct once lodged there, remarking only that o portion of the duct is specialized as a sphincter.

Evans and Damant (1928) considered the pneumatic bulb of roach, tench, bream, carp, and minnows to be adapted to pumping air into the gas bladder. Jones and Marshall (1953) remarked that this is probably one of its functions in view of Plattner's work (1941). When Plattner cut the vagus branch running to the sphincter, air taken at the surface accumulated in the intestine and did not pass into the bladder. However, nothing in the ducts of the sardine and anchovy resembles the strong sphincter containing numerous striated muscle fibers described by Evans and Damant. On the other hand, certain authors have observed, in some of the clupeoids in which the duct arises from the end of the blindsac, that the duct has a "beady" appearance. A few also noted that this seemed to be variable. I have observed this condition in the lower half of the duct in the sardine, but not in the anchovy. These observations suggest that the duct carries on a sort of peristaltic action, at least in the sardine. Owing to the orientation of the lower septa in the sardine, muscular contraction of the wall would tend to expel foreign matter into the stomach, and a peristaltic action could possibly move gas away from the solid matter and pump it toward the bladder.

It is hardly necessary to comment on the function of the duct as a passage for the escape of gas. Expansion of gas in the bladder and relaxation of the muscularis of the duct would suffice to accomplish this.

THE GAS-BLADDER WALL

Fänge (1953) discussed at some length the differences in the terminologies that have been applied to the tissue layers of the gas bladder in fishes. He points out the need for a uniform and well-defined terminology, and suggests that the terms commonly used to designate the different tissue layers of the vertebrate digestive tube be applied to the gas bladder.

The four tissue coats of the digestive tract are mucosa, submucosa, muscularis externa, and serosa. The mucosa is further subdivided into epithelium, lamina propria, and muscularis mucosa. In applying this terminology to the gas bladder of euphysoclists, Fänge concluded that the muscle layer present can best be characterized as muscularis mucosa, and that there is, in place of the muscularis externa, a thick layer of connective

tissue. For the latter, he considered the term "tunica externa" more appropriate than "muscularis externa."

From his own observations on *Clupea harengus*, as well as those of Maier and Scheuring (1923), Fänge concluded that in clupeids the bladder is composed of an epithelium, an inner compact connective tissue layer, probably containing muscles, and an outer, voluminous connective-tissue layer. He further concluded that in physostomes, as in euphysoclists, the smooth muscles of the bladder wall may be characterized as muscularis mucosa.

Using Fänge's terminology, the tissue layers in the gas bladder of the sardine and anchovy were readily defined as epithelium, muscularis mucosa, tunica externa, and serosa. In both species, the epithelium is simple squamous, and, at certain points, elevated in longitudinal folds. The well-developed muscularis mucosa shows different specializations in the two species. Since both transverse and longitudinal elements are present, the terms "transverse smooth muscle" and "longitudinal smooth muscle" were applied to the accompanying illustrations. The tunica externa, a loose fibroelastic connective tissue, covers the whole bladder. It varies somewhat in thickness, but formalin fixation produced too much shrinkage and distortion to permit an accurate description. It can be stated, however, that no muscle fibers are present, and that the layer is but sparsely penetrated by blood vessels. The blood vessels supply a thin bed of capillaries, which vascularizes the mucosa, located between the muscularis mucosa and tunica externa. The serosa overlays the tunica externa, except on the dorsal side of the bladder. No submucosa could be distinguished in either species.

The detailed descriptions of the bladder wall that follow are based on sections made through various parts of the bladder in the sardine and anchovy. The tunica externa and serosa are not discussed.

Sardinops caerulea

In figure 10, drawings of four cross sections of the gas-bladder wall of the sardine are shown. All are enlarged × 680. The plane of each section is indicated on the drawing of the bladder. Sections D and E, through the pneumatic duct, are placed here for easier comparison with the bladder wall.

Sections A, B, and C are taken, respectively, through the anterior constricted portion, the region of greatest expansion, and the posterior tubular portion. Noticeable in all sections is the connective-tissue matrix in which the individual muscle cells are embedded. This tissue has the same composition as, and is continuous with, the lamina propria beneath the epithelium and the thickening between the muscle and vascular layers. Fänge, describing the bladder wall in euphysoclists, observed that there were sheets of connective tissue running through the muscle layer. He suggested, since these were probably continuous with the dense connective tissue on either side of the muscle layer, that the term "lamina propria" could be applied to all of the mucosa except the epithelium. The mucosa ranges in thickness from 15 or 20 microns in section B to 45 or 50 microns in section C.

In section A, the transverse muscularis is relatively thin, but the wall is considerably thickened by the connective tissue of the mucosa and by the vascular layer. The epithelium is raised in longitudinal folds about 20 or 30 microns high. The bladder lumen here, incidentally, is 160 or 170 microns in diameter. This is the only part of the bladder in which capillaries were definitely present in the thick connective tissue of the mucosa as well as in the vascular layer outside of it. Some of them are adjacent to and even appear to penetrate the muscular layer; however, none were detected in the connective tissue between the muscle coat and the epithelium.

The form of the epithelial cells of the longitudinal folds is difficult to ascertain. Some authors have described the cells as being low columnar in clupeoids they have examined. In my preparations, they varied in shape from squamous to columnar. Cells in the depressions between folds tended to be cuboidal, while those at the apex of the folds tended to be columnar. Along the sides of the folds they seemed to be squamous, but it is here that the greatest variation occurs, since these surfaces are complicated by some folding and branching. It is my impression that the cells are actually squamous over this whole region, as they are in the rest of the bladder; that they are compressed in the depressions and projected on end at the narrow peaks. This opinion applies to all regions exhibiting epithelial

olds in the gas bladders of the sardine and nchovy.

In section B, typifying the expanded portion of ne bladder, the epithelium is a simple pavement f squamous cells, and the muscle coat is composed f transverse fibers.

A little anterior to the insertion of the pneuatic duct, occasional longitudinal muscle fibers ppear within the transverse muscle layer. These ncrease in number posteriorly to become domiant a short distance behind the pneumatic duct section C). There are, however, occasional roups of fibers having an oblique alinement. hese merge gradually into the adjacent longiudinal cells, indicating perhaps that the fibers re "woven" to some extent. As the diameter of he bladder decreases towards its posterior end, he muscle cells become thinner, and the epitheium is again raised in longitudinal folds. Also, hin, tranverse muscle fibers appear among the ongitudinal fibers.

Maier and Scheuring (1923) described the dense ayer of tissue beneath the epithelium in *Clupea arengus* as a lamellar connective tissue composed f longitudinal fibers. They suspected the presnce of muscle fibers, at least in the posterior part f the bladder, but could not make a conclusive tatement, explaining that their specimens had een improperly fixed.

The terminal portion of the bladder, and its pening to the exterior, will be described in detail n page 523.

Engraulis mordax

The gas bladder of the anchovy differs from hat of the sardine in having a well-developed ransverse septum which divides it into a small nterior and a large posterior chamber, and in aving, instead of an opening to the exterior, a very small caecum at its posterior tip.

Figure 11 is a series of sections of the bladder wall reproduced at different magnifications and in different planes. The tissue layers in the anchovy are basically the same as in the sardine. The most noticeable difference is that transverse muscle fibers predominate throughout. Also, the tunica externa is somewhat thicker and more dense around the middle, constricted portion of the bladder.

Section A, at a magnification of × 150, illustrates the cartilage-encased capillary that extends to the cranium. The outer diameter of the cartilaginous sheath is about 200 microns and the diameter of the capillary lumen is about 20 microns. It is probable that the capillary, composed of epithelium, a very thin muscularis, and a thin connective tissue layer, has contracted away from the inner wall of the cartilaginous tube, and that the lumen of the capillary, therefore, has a greater diameter in the living animal. Of course, section A is also typical of the more anterior, bifurcated portion of the capillary and its cartilaginous sheath. The only differences noted were slight variations in diameter. Except for a slight difference in size, the structure of the paired anterior capillaries is the same in the sardine though no description was given.

The anterior end of the bladder (not illustrated) has epithelial folds and a slightly thickened vascular layer, as in the sardine.

The mucosa of the bladder wall varies in thickness from 12 microns to about 50 or 60 microns. Both the anterior and posterior chambers are thin-walled with transverse muscle fibers and squamous epithelium. This is illustrated only for the posterior chamber (section D). In the constricted portion of the bladder, between the duct insertion and the septum, the mucosa is about five times as thick as elsewhere. This is illustrated in section C, a longitudinal section at the same magnification as section D. The increase in thickness is due in part to a marked thickening of the transverse muscle layer and in part to the addition of a layer of longitudinal muscle fibers between the transverse muscle layer and the tunica externa.

Section B is a longitudinal view of the septum at a magnification of × 45. The main component of the septum is a bundle of closely packed smooth-muscle fibers encircling its centrally located opening. In the preparation sketched, the septum is about 300 microns thick. Beneath the septal ring, there are muscle and connective-tissue fibers running in several directions, but it is clear that many of the muscle fibers of the longitudinal layer described above turn inward to the base of the septal ring itself. The dark sheets penetrating the ring vertically represent definite thickenings of connective tissue. Although no muscle fibers were detected within them, these dark sheets are in some instances clearly continuous with bundles of longitudinal muscle fibers, and are

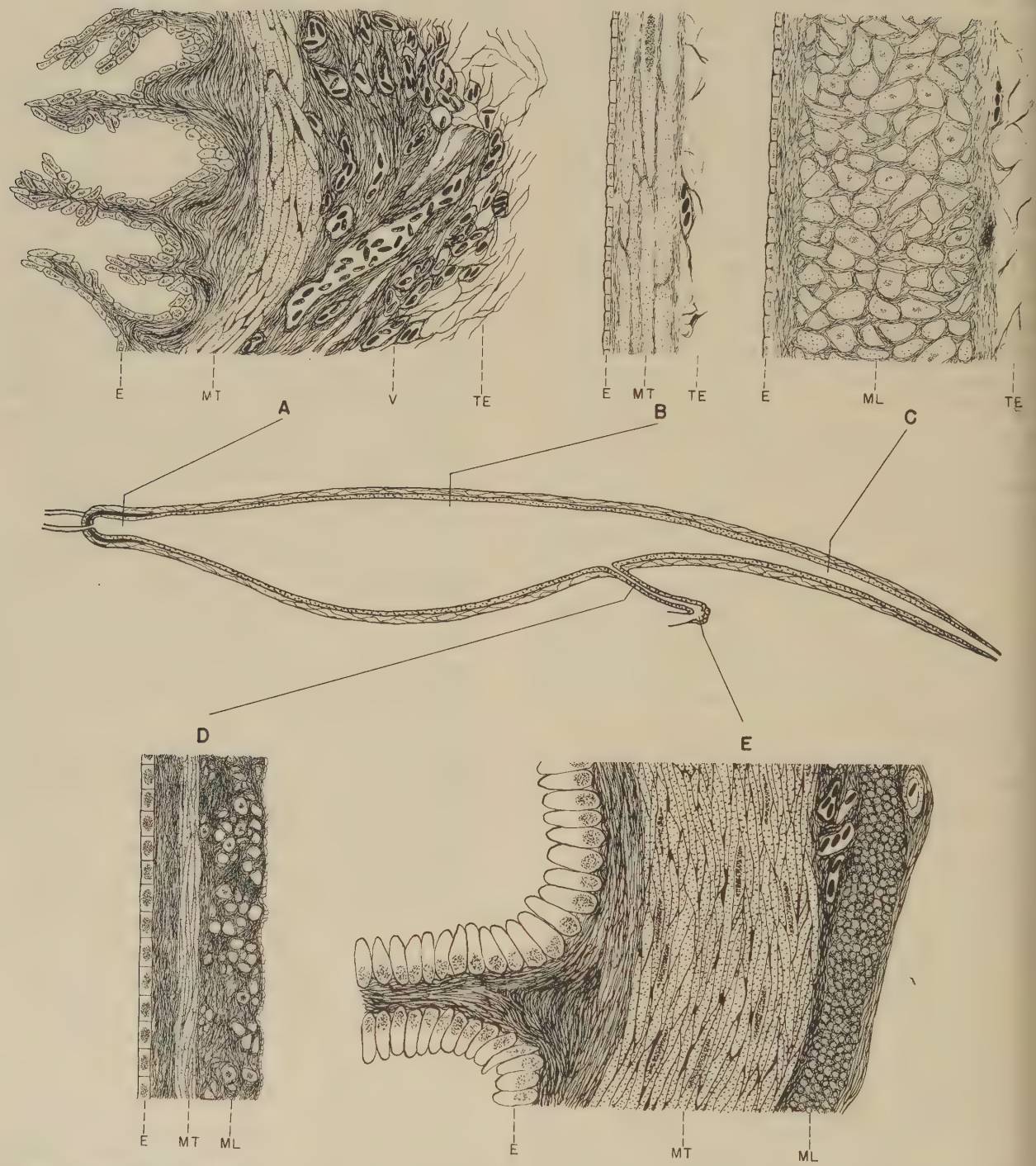

FIGURE 10.—Selected cross sections of the gas-bladder wall of *Sardinops caerulea* (195 mm. standard length). A. Anterior tubular portion. B. Expanded portion. C. Posterior tubular portion. D. Wall of the upper part of the pneumatic duct. E. Wall of the lower part of the pneumatic duct. Magnification × 680. *E*, epithelium; *ML*, longitudinal smooth muscle; *MT*, transverse smooth muscle; *TE*, tunica externa; *V*, vascularis.

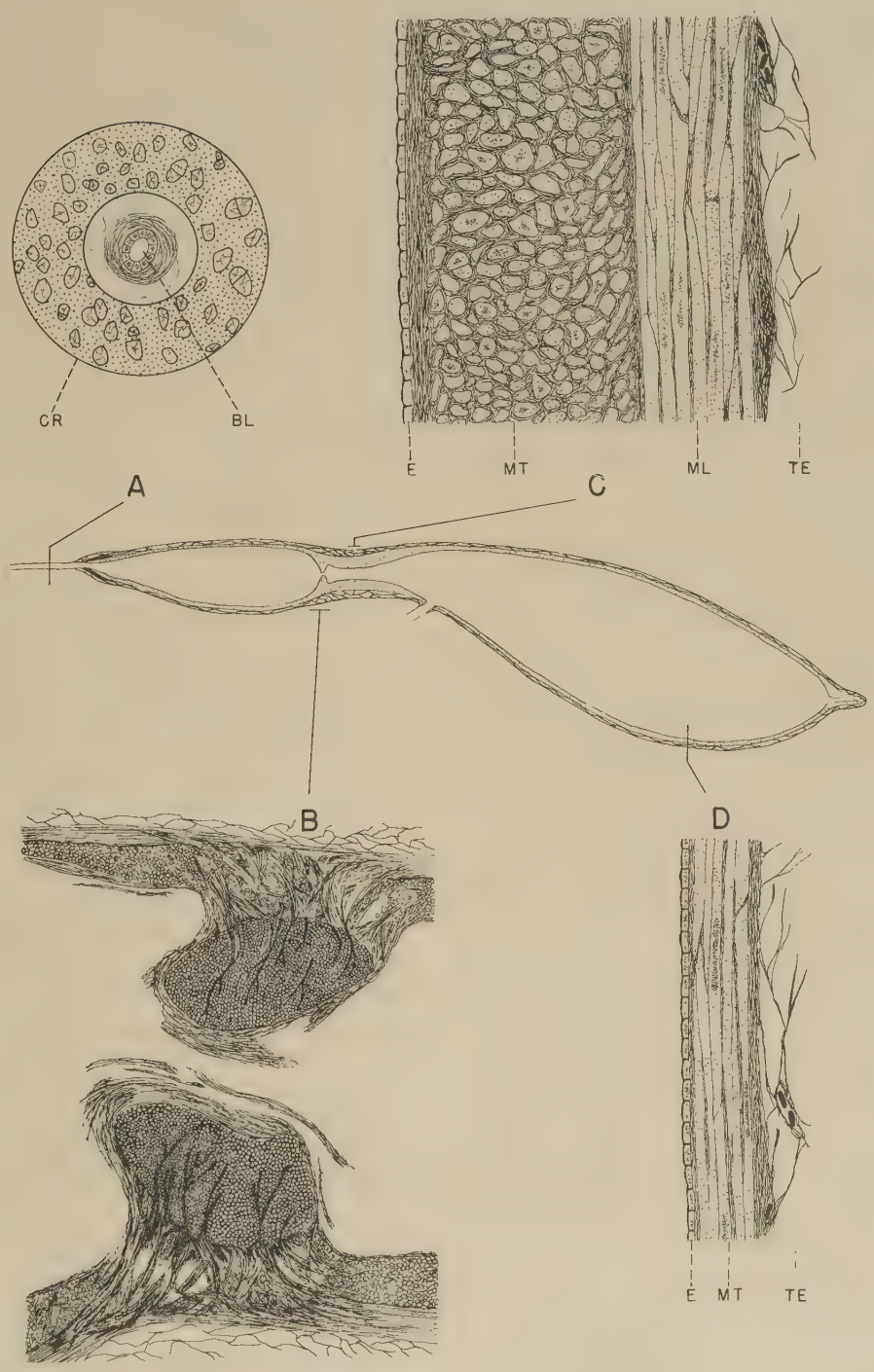

FIGURE 11.—Selected sections of gas-bladder wall of *Engraulis mordax* (145 mm. standard length). A. Cross section of the median capillary (× 150). B. Longitudinal section of septum (× 45). C. Longitudinal section from the middle, tubular portion (× 680). D. Cross section from the posterior chamber (× 680). *BL*, lumen of gas bladder; *CR*, cartilage; *E*, epithelium; *ML*, longitudinal smooth muscle; *MT*, transverse smooth muscle; *TE*, tunica externa.

probably tendons. The squamous epithelium is raised in longitudinal folds over both faces of the septum and along the thickened part of the bladder wall behind it. This accounts for the apparent irregularity in the thickness of the connective tissue over the inner surface of the muscle layer in section B.

Functions of the gas-bladder wall

In the sardine and the anchovy, the epithelium is primitive throughout, but the muscularis mucosa shows some degree of specialization. It is possible but not very probable that the epithelial folds in the bladders of these two species constitute gas-secreting organs. The epithelial folds differ in four essential ways from the well-developed gas glands to be found in physoclistous fishes, as follows:

1. The epithelial cells in the folded regions are squamous as in the rest of the bladder. In well-developed gas glands the epithelial cells are highly specialized, usually columnar, and larger than elsewhere in the bladder.

2. The epithelial folds are not complex. Although there may be irregularities in a given fold, there is no evidence of fusion between major folds. In well-developed gas glands there is usually greater complexity, ranging from scant fusion between folds of simple epithelium to fusion in stratified epithelium so complex that numerous glandular pits are formed.

3. There are no vascular specializations adjacent to the epithelial folds. The concentration of capillaries at the anterior end of the bladder in the sardine and the anchovy evidently represents a convergence of capillaries from extensive areas of the bladder wall to larger vessels of the venous system. The muscularis mucosa intervenes between the capillary layer and the epithelium. In physoclists, blood vessels extend directly to the glandular epithelium from the rete mirabile.

4. The folds are present only in constricted regions of the bladder, i. e., the most anterior portion in both species, the posterior, tapered portion in the sardine, and the middle, constricted portion in the anchovy. Among the physoclists, gas glands tend to occur in the anterior part of the bladder, but usually are not located in constricted regions.

Considering all four points, it seems quite possible that the folds in the sardine and anchovy represent nothing more than crowding of the epithelium in regions that are usually constricted, but capable of considerable expansion, as is the case in the teleostean digestive tract. Bridge (1904, pp. 262–263), in discussing the digestive tract of fishes, noted that "the simple longitudinal folds which are sometimes found in the oesophagus, stomach, and rectum, often disappear on distension, and probably merely provide for the enlargement of these cavities. . . ." Even in the absence of specialized glands, however, secretion by the epithelium at a very low rate is quite possible. Evans and Damant (1928) have demonstrated this in certain cyprinoids that do not have gas glands.

The muscularis mucosa is well developed in both species, but highly specialized only in the middle, constricted portion of the bladder in the anchovy. Fänge (1953) demonstrated that its function is primarily concerned with secretion and absorption of gas in the euphysoclist bladder. The opening of the oval sphincter is accompanied by the relaxation of the muscularis mucosa over the whole resorptive portion of the bladder, and its contraction over the whole secreting portion. Conversely, activation of the gas gland is accompanied by relaxation over the entire secreting portion and contraction over the entire resorptive portion. Evans and Damant (1928) and Franz (1937), on the other hand, have shown that the gas-bladder pressure in the Ostariophysi is slightly greater than atmospheric pressure. Jones and Marshall (1953) remarked that this pressure, which is supposed to keep the wall at a suitable tension for the functioning of the Weberian mechanism, is probably maintained by the tonic contraction of the bladder. Thus, the muscular coat of the bladder may perform different functions in different groups. There is nothing in the structure of the clupeoid bladder, however, that indicates the need for either of the above functions. Without further investigation, it can only be supposed that the tonus of the muscularis changes to maintain the wall at a constant tension against changes in volume.

The heavy development of the muscularis in the constricted portion of the bladder in the anchovy is indicative of some functional specialization. The ring of transverse muscles in the septum undoubtedly serves to close the central opening of that structure, and the longitudinal muscle fibers attached to it probably serve to expand the open-

g. Along with this, the presence of both transrse and longitudinal muscle coats, particularly tween the septum and pneumatic-duct orifice, ggests a peristaltic pumping mechanism capable shifting gas from one chamber to the other.

THE POSTANAL OPENING IN *SARDINOPS CAERULEA*

Although the relative positions of the three penings behind the anus have been repeatedly oted for clupeoid species having a postanal openng of the gas bladder, there does not seem to be detailed histological description of this region. nvariably the genital duct opens directly behind ne anus, and the urinary duct directly behind he genital duct with the gas bladder opening to he left of the genital and urinary pores. Ridewood (1891) noted slight individual variation in he relative position of these openings in one pecies, but the bladder opening was always to he left of the others.

Tracy (1920 a), conjecturing on the functional relation between the gas bladder and its ranial vesicles, conditioned his hypothesis on whether the postanal opening of the bladder was ontrolled by a sphincter. Later Maier and Scheuring (1923), after examining this region istologically in *Clupea harengus*, concluded that he muscularis of the bladder wall near the openng could not function as a sphincter because it s composed almost entirely of longitudinal fibers. They attributed the function of closing the gasbladder pore to a compact mass of striated muscle ibers, which they described as forming a ring around the whole anal field, i. e., enclosing all four openings. The sphincter, thus labeled in their drawing, corresponds in size and position to the protractor analii (or retractor ischii) of the sardine. According to Whittaker,[3] this is a pair of cylindrical muscles which arises from the pelvic girdle, extends along each side of the midventral body line, and passes on either side of the anal opening to insertions on the first haemal spine. This pair of muscles, by virtue of its known function in protracting the anal fin and retracting the pelvic fins, could not serve efficiently to close the ducts opening in the anal region. Furthermore, it does not seem logical that these four openings, each serving a different organ system, would be controlled as a unit.

Figure 12 is a ventral view of the duct openings of a sardine 240 mm. standard length that was reconstructed from a serial section through this region. All four ducts terminate in a depression, or subcutaneous pit, located just anterior to the first haemal spine. The mouth of the pit is diamond shaped, 3 or 4 mm. long and 1.5 or 2 mm. wide. Anteriorly, the anus and the surrounding mucous membrane of the pit are flush with the body wall along the midventral line. Posteriorly, the pit deepens to the first haemal spine. Extending back from the rectal portion of the intestine is a median ridge that bears the genital and urinary openings. The former is a longitudinal slit, and the latter a small, round pore. This pore actually opens from the urinary bladder located within the ridge. The posterior margin of this ridge, or urogenital papilla, is formed by the recurved urinary duct descending into the primitive urinary bladder. The papilla, which protrudes almost to the level of the mouth of the pit, is a muscular structure serving as a sphincter for the genital and urinary openings.

The margin of the pit is lined with overlapping scales, which produce, along with the underlying mucous membrane, a narrow horizontal lip, or shelf. From this shelf, the walls on each side converge dorsally to their junction with the base of the urogenital papilla. The gas-bladder terminus appears as a small folded patch, or papilla, on the sloping left wall.

Figure 13 is a series of selected cross sections through the subcutaneous pit. The plane of each section, labeled by its number in the series, is indicated in figure 12. In each section, a small portion of the lip forming the margin of the pit is visible. The gas bladder, urinary duct, genital duct, and subcutaneous pit are labeled. The relative position of structures refers, of course, to their orientation in the fish.

Section 1, just behind the anus, shows the subcutaneous pit beginning to deepen, most of the space being occupied by the broad urogenital papilla. The genital duct is centered in the papilla. The urinary duct, running somewhat obliquely, is immediately above it, and the gas bladder is a discrete tubule to the left of the

[3] Whittaker, W. R., 1932. Anatomy of the North American pilchard and its bearing on the genetic relationships and nomenclature. Unpublished—submitted as M. A. thesis at University of British Columbia.

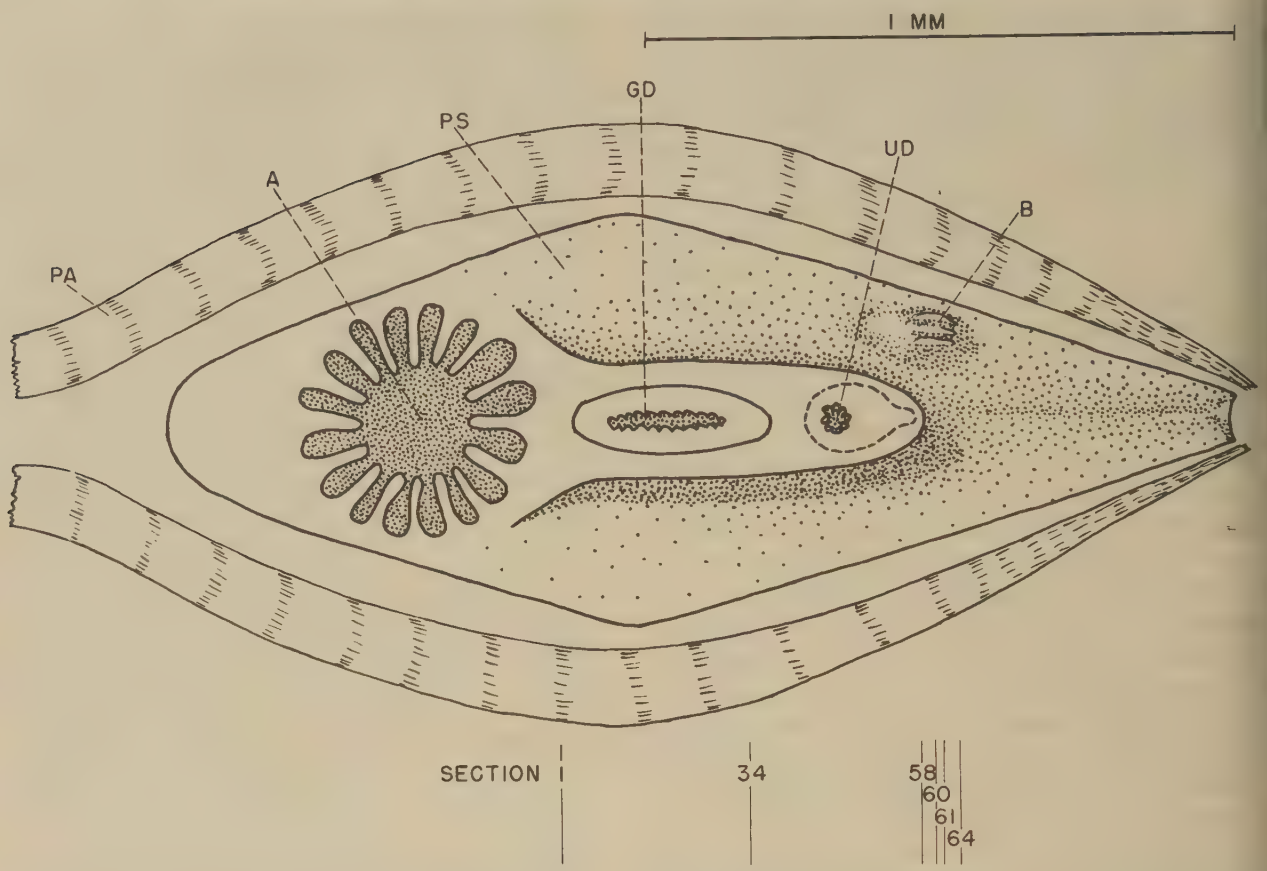

FIGURE 12.—Ventral view of postanal region of *Sardinops caerulea* (240 mm. standard length). The lines below the drawing mark the verticals of the sections shown in the next figure. *A*, anus; *B*, gas bladder; *GD*, genital duct; *PA*, protractor analii; *PS*, subcutaneous pit; *UD*, urinary duct.

urinary duct. The irregularity of the bladder lumen is apparent. The wall of the tube is composed of very small muscle fibers, mostly transverse. The darkly stained region just under the epithelium is dense connective tissue, constituting the lamina propria. Vascular elements were not present here. The epithelium is a simple layer of small cuboidal cells, quite different from the lining in other parts of the bladder. In fact, the epithelium changed abruptly less than a millimeter anterior to this section.

The urogenital papilla is actually a massive sphincter. It is composed of dense connective tissue permeated by numerous capillaries and small, striated muscle fibers running in various directions, and is covered externally by the mucous lining of the pit.

In section 34, the genital duct has already opened to the exterior, and the urogenital papilla now projects freely into the subcutaneous pit. The urinary duct is entering the muscular papilla, and the nerve from the right side of the papilla runs dorsad, eventually penetrating the region of the body muscles. The gas-bladder tubule is descending obliquely into the lining of the subcutaneous pit. The lamina propria is dwindling, and disappears suddenly a few sections later. At the same time the muscular wall ends, and the lumen of the tubule, bounded by the cuboidal epithelium, enters the mucous lining of the pit, becoming highly irregular.

In section 58, the gas-bladder lumen is visible as several small tubules in folds of a prominent papilla on the left wall of the subcutaneous pit. The nerve from the left side of the urogenital papilla passes dorsad close to the folds of the gas-bladder papilla. The terminal portion of the urogenital papilla projects freely into the pit.

Sections 60, 61, and 64, further illustrate the structure of the gas-bladder papilla. In this

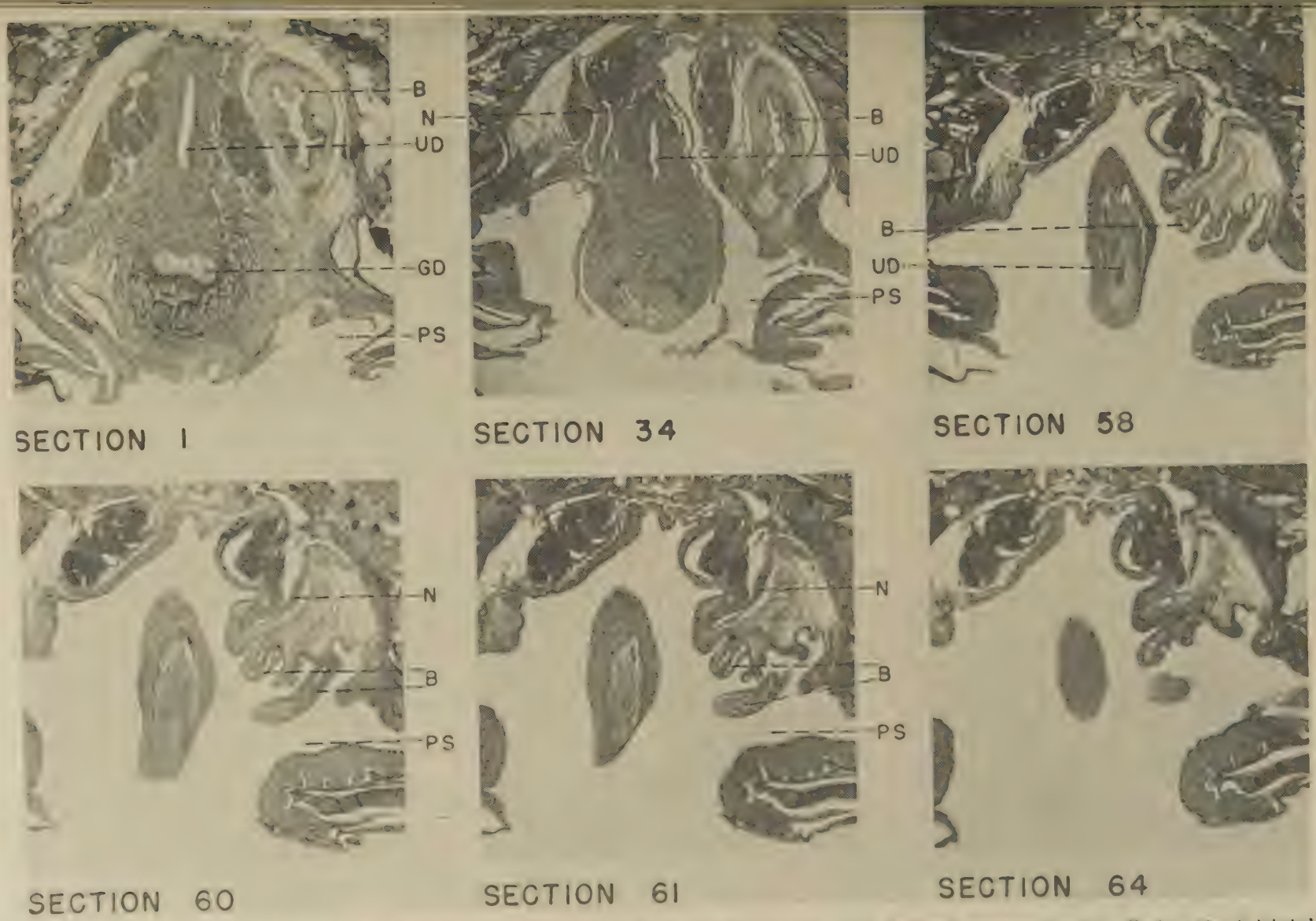

FIGURE 13.—Photographs of selected cross sections through the postanal region of *Sardinops caerulea* (240 mm. standard length). Each section is labeled by its number in the series, and the plane of section is indicated in figure 12. Magnification is × 40. *B*, gas bladder; *GD*, genital duct; *N*, nerve of urogenital sphincter; *PS*, subcutaneous pit; *UD*, urinary duct.

specimen, the papilla bears three short longitudinal folds, or lappets. The lateral and medial of these lappets contain a number of very small but distinct tubules lined by cuboidal epithelial cells. When traced, the spaces in each lappet were found to be discontinuous segments of one lumen. The lumen in the medial lappet, readily established as a continuation of the gas bladder, is a narrow and tortuous tubule. Three very small but definite, and apparently isolated, openings to the exterior were discerned. Continuity with the gas bladder could not similarly be established for the tortuous tubule in the lateral lappet; nor could openings to the exterior be detected. I surmise, however, that this tubule is also continuous with the gas bladder, the connection being fortuitously obscured by folding of the highly flexible papilla.

It is apparent from this series of sections that the intestine, genital and urinary ducts, and gas bladder tubule remain separate to their terminal openings. The genital and urinary openings are controlled by one massive sphincter, but no specialized sphincter occurs at the gas-bladder opening. As Maier and Scheuring remark, it is doubtful whether the scanty muscularis in the last few millimeters of the bladder wall constitutes an effective sphincter.

The structure of the gas-bladder papilla and its location in the mucous lining of the subcutaneous pit suggest that the papilla functions mechanically as a kind of check valve. Expansion of the gas in the bladder would distend the lappets and dilate the openings to the exterior, permitting gas to escape, whereas compression by the water outside would cause the papilla, its lappets, and included lumina to collapse upon themselves and effectively seal the openings to the exterior. A mechanism of this sort would be more sensitive and efficient and less complicated than a muscular sphincter in maintaining a relative pressure equality. When gas pressure exceeds water pressure, distension of the papilla permits gas to escape. When water pressure exceeds gas pressure, collapse of the papilla prevents water from entering the bladder. Of course, if the water pressure becomes too much greater than the gas pressure, it is possible that the papilla would rupture. Presumably, the fish possesses a reflex mechanism that would curtail movements causing too great a differential in this direction.

THE POSTERIOR CAECUM IN *ENGRAULIS MORDAX*

The posterior caecum of the bladder in the anchovy is about 2 mm. in length and 1 mm. in diameter, and its posterior tip turns slightly upward. The whole structure is covered by a relatively thick tunica externa. Figure 14 is a drawing of the caecum reconstructed from a series of cross sections, and figure 15 shows six selected sections from this series. The plane of each section, labeled by its number in the series, is indicated in figure 14. The last three photographs in figure 15 are at greater magnification than the first three.

Section 1 shows the mouth of the caecum at the upper end of the bladder. Shortly anterior to this, the muscularis of the bladder wall begins to thicken, and the epithelium begins to develop folds. The bladder lumen ends a few sections beyond.

Section 46 shows the typical form of the caecum. The tunica externa and muscularis are each about 150 microns thick. Although the muscularis is predominantly transverse, there are scattered longitudinal fibers. The lumen of the caecum, about 600 microns in diameter, is filled by very tall, branched epithelial folds. The latter appear in dissection as close-packed radial lamellae.

Posteriorly, the caecum and its lumen diminish in diameter until the lumen turns sharply upward and opens into a small, thin-walled chamber situated in the tunica externa (section 234). This chamber, which may be called the membranous chamber of the posterior caecum, is about 300 microns in both diameter and length, and is unequally bilobed. The caecal lumen opens into the larger segment, on the left in the fish. The two segments are about the same length and communicate by a small, transverse opening.

The size of the caecum makes a detailed study of the structure by dissection impossible. Microscopic examination of several specimens indicated that the membranous chamber has some range of expansion. In some, the chamber appeared to be 400 or 500 microns long.

Sections 237, 243, and 245 illustrate the detailed structure of the membranous chamber. The longitudinal folds of the caecum extend freely into the larger (left) segment to form a rosette occupying most of the available space. These folds, or lamellae, do not extend into the smaller (right)

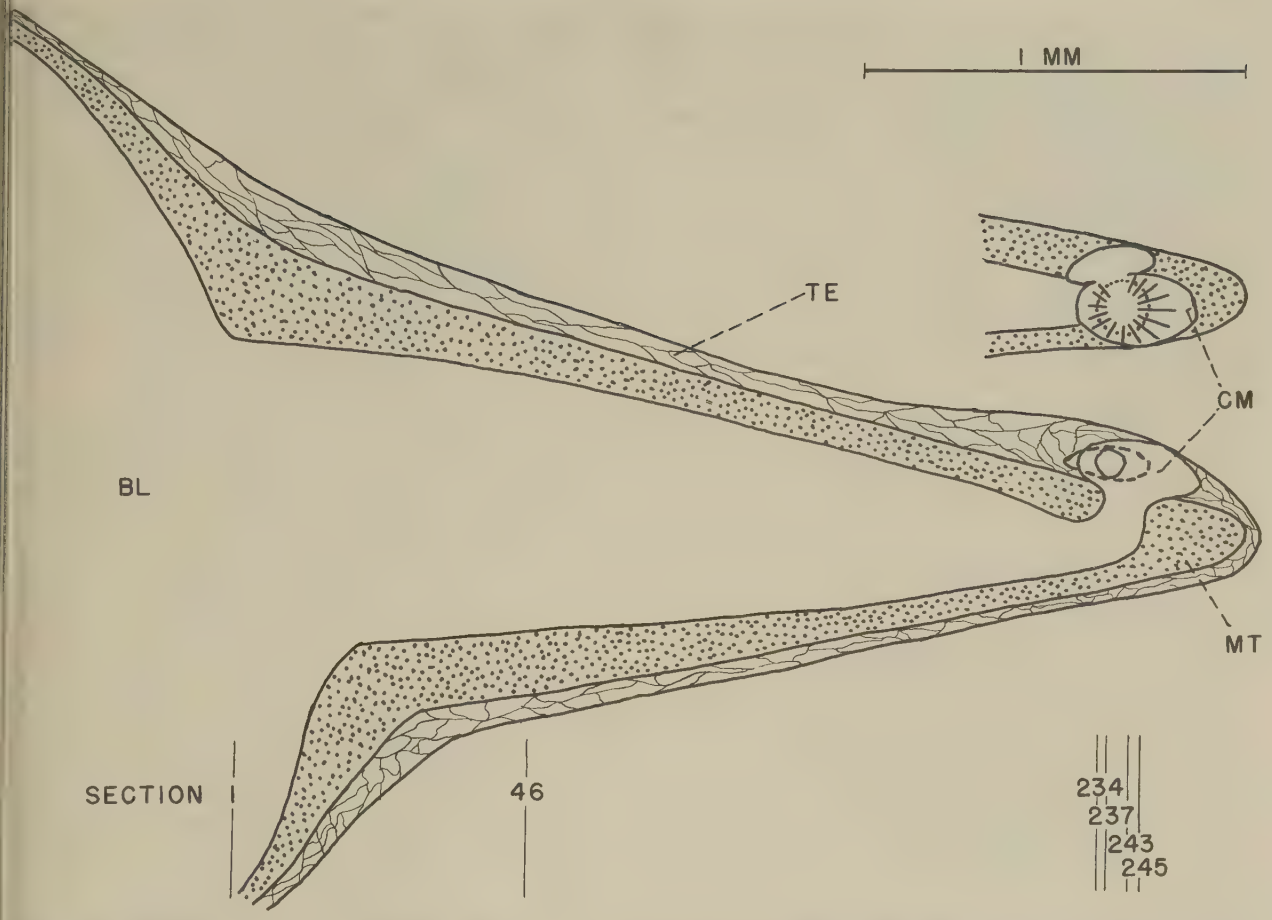

FIGURE 14.—Reconstruction of the posterior caecum of the gas bladder in *Engraulis mordax* (145 mm. standard length). The posterior tip is shown in dorsal as well as lateral view. The vertical lines below the drawing mark the verticals of the sections shown in figure 15. *BL*, lumen of gas bladder; *CM*, membranous chamber of posterior caecum; *MT*, transverse smooth muscle; *TE*, tunica externa.

egment, nor are they very prominent in the anerior part of the larger segment.

The wall of the chamber, about 6 microns thick, ontains three tissue layers: epithelium, connecive tissue of the mucosa, and fibrous connective issue of the tunica externa. In other words, the muscularis is absent.

The epithelium in the caecum and in its membranous chamber is histologically the same as in other parts of the bladder; that is, the cells appear o be squamous, although they are distorted by he folding of the lining. The folds here, incidentally, are higher and more definite in form than are those described for other parts of the bladder. They are branched to some degree, but are not fused. Capillaries could not be found in the connective tissue of the folds; however, a heavy concentration of vascular elements passes through the tunica externa and enters the muscularis around the base of the membranous chamber. This is best shown in section 234. It does not constitute a capillary mass, or rete mirabile.

It is difficult to assess the function of the posterior caecum without further information. The extremely well-developed folds which extend from the caecum into the membranous chamber suggest, because of the increased surface thus developed, either a secretory or an absorptive function; however, the epithelial cells display no secretory specialization. Furthermore, there is no evidence of the vascular specializations that normally accompany well-developed secretory or absorptive structures. The absence of the muscularis, as well as the position of the organ on the dorsoposterior part of the bladder wall, are reminiscent of the oval present in many physoclistous fishes. The

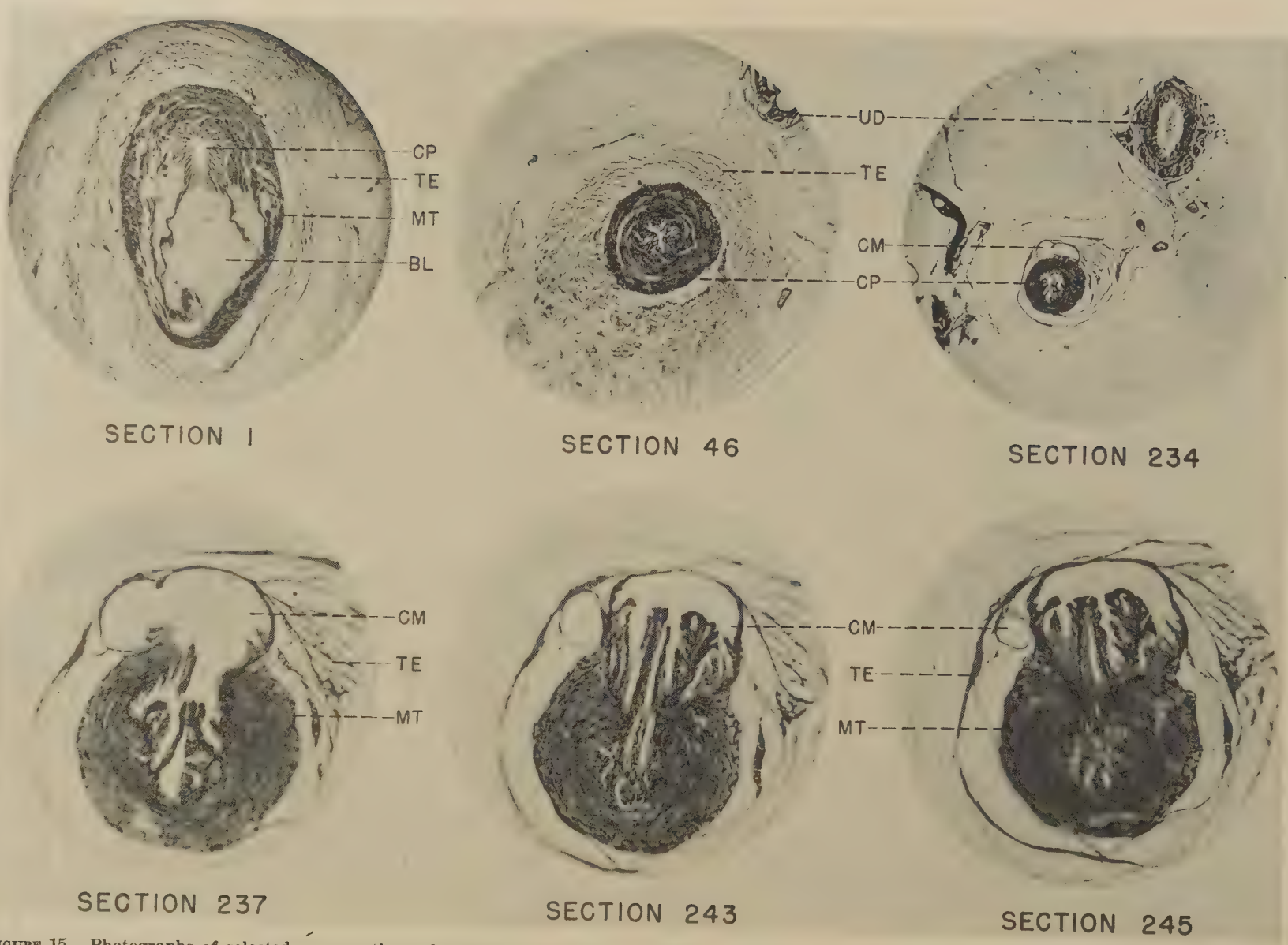

FIGURE 15.—Photographs of selected cross sections of posterior caecum of gas bladder in *Engraulis mordax* (145 mm. standard length). Each section is labeled by its number in the series, and the planes of section are marked in figure 14. Magnification is × 30, for sections 1, 46, and 234, and × 135 for sections 237, 243, and 245. *BL*, lumen of gas bladder; *CM*, membranous chamber of posterior caecum; *CP*, posterior caecum; *MT*, transverse smooth muscle; *TE*, tunica externa; *UD*, urinary duct.

muscular caecum in the anchovy could be analogous to the sphincter that opens and closes the oval. Should the structure actually be a diffusion membrane, it is a strikingly minute and atypical one.

A third possibility is that the caecum, or more specifically, its membranous chamber, functions as some sort of a sensory receptor in a reflex mechanism that controls volume changes in the bladder. However, no specialized sensory elements were found.

DISCUSSION

The relation between the gas bladder and the pars superior of the ear in clupeoids has provoked considerable conjecture. Among 19th and early 20th century investigators, the controversy was largely over structure, but now that the anatomical details are well defined, it has shifted entirely to function.

Association of the gas vesicles with the pars superior rather than the pars inferior of the ear caused some skepticism in assigning an auditory function to the mechanism. In all other groups of fishes, and for that matter in all other vertebrates, the pars inferior is the seat of auditory perception, while the pars superior is the seat of the sense of equilibrium. As Wohlfahrt (1936) pointed out, this difference is also reflected in the nerve tracts of the auditory nerve in all vertebrates. The axis cylinders of the ramus vestibularis (ramus anterior VIII) are always a little thicker than those of the ramus cochlearis (ramus posterior VIII). He found this to be the case in *Clupea pilchardus*, with the exception that the axis cylinders of the ramulus utriculus anterior have the same diameter as those of the ramus posterior VIII. For this and other anatomical reasons, he believed the macula utriculus anterior to be an auditory receptor.

Wohlfahrt, after considering at great length the functional theories developed by Evans (1932, 1935) and Tracy (1920 a), stated that the former investigator based his ideas on erroneous histological interpretations, while Tracy considered an auditory function unreasonable for both physical and biological reasons. Suffice it to say here that, in one paper, Evans designated the pars inferior as the seat of auditory perception, and considered the gas-bladder connection to the pars superior and the specializations of the recessus utriculus as part of a hydrostatic reflex mechanism to maintain an optimum tension within the membranous labyrinth (actually pressure exerted by the sacculus against the thin plate covering the auditory fenestra). Later Evans proposed the pars superior as the auditory receptor, but in both instances he described an improbable path of conduction for the transmission of sound waves. He also described the sensory elements of the recessus utriculus incorrectly. Tracy proposed a hydrostatic function for the mechanism, believing that bone and liquid in the cranium would be more efficient sound conductors than gas in the cranial vesicles.

Wohlfahrt assigned a double function to the pars superior. He presents convincing evidence of auditory reception in the macula utriculus anterior, but surmises that the lapillus still performs equilibrium functions. He considers the bony gas-filled bullae to be resonators that vibrate uniformly to sound waves, activating the prootic membrane.

This hypothesis can, perhaps, be carried one step further. Quite possibly the gas-filled bony chamber has a natural period of vibration and responds to a given acoustic frequency or range of acoustic frequencies in the environment. In this case, the pars superior would perform only an accessory auditory function, with the pars inferior functioning as in most other fishes.

The mechanism unquestionably perceives the same kind of stimuli in all clupeoid species, since in all so far investigated the lapillus, maculae utriculi, prootic fenestra, and prootic membrane have the same structural relation. The bony bullae that contain the gas vesicles, however, differ in size and shape in the sardine, anchovy, and other species. It is possible that such differences indicate specifically different ranges or degrees of perception, but this can only be determined by laboratory experiments. The presence or absence of pterotic bullae, for example, would not alter perception in kind, but might well alter it in degree.

What role this sensory mechanism has in the behavior of the fish is unknown. As an accessory auditory receptor it would probably increase the frequency range, and perhaps the sensitivity of hearing. The Ostariophysi and Mormyridae, which have other types of ear-gas bladder connections, perceive higher frequencies than fishes that

lack such connections (Griffin 1950). If the differences in the size and shape of the bullae produce different ranges and degrees of perception, some species are better equipped than others. On the other hand, it is possible that the mechanism plays some role in recognition among individuals of a species.

Very little is known of the functions of the gas bladder in clupeoids. Since it is present, the bladder must, of course, function as a hydrostatic organ; however, to what extent it serves to put the fish in hydrostatic equilibrium with its environment is not known. Magnan (1929) measured the density of *Clupea harengus*, but Jones and Marshall (1953) considered his figure surprisingly high for a pelagic fish with a well-developed gas bladder. From Magnan's data they calculated a sinking factor in the range of demersal fishes, and suggested that the determinations need repeating. They further noted that the herring (quoting Reay et al. 1943; Stansby and Lemon, 1941) shows considerable seasonal variation in fat content, and that more must be known about the tissue density of this fish before correlations between the size of the gas bladder and the fat present in the tissues are attempted. Since the density of fat is less than that of sea water, large accumulations of fat at certain seasons would reduce the bladder volume required to put the fish in hydrostatic equilibrium. Of course, concurrent changes in other organs, such as the gonads, may present a further complication.

The presence of well-defined layers of smooth muscle in the gas bladders of the sardine and anchovy and the different states of contraction noted in preserved specimens are indicative of an ability to alter the form and volume of the organ. Such differences, if necessitated by density changes in various tissues, could be sustained by changes in the muscle tonus of the bladder wall.

If buoyancy is maintained at a constant level during vertical movements, the gas-bladder volume must be kept constant. This can only be accomplished by adding gas during descent and discharging it during ascent. No matter what role the bladder plays in the behavior of the fish, its volume must be maintained within some limits to avoid extreme distortion and consequent loss of control by the fish of its vertical movements. It has been shown for some other species (Jones 1952), that there is, undoubtedly, a vertical range within which the fish can move comfortably without changing the gas content of the bladder. Beyond this range, the rate of vertical movement would be determined by the rate at which gas could be added or expelled.

In the sardine and anchovy, the only possible sources of gas are the pneumatic duct and the epithelium. Air swallowed at the surface could be transmitted to the bladder through the duct, but, as Jones and Marshall point out, glands are the only possible source of gas when the fish is at a distance from the surface. In these two species, the construction of the pneumatic duct would facilitate the movement of gas toward the bladder while excluding solids, and the epithelium, lacking gas glands, may be capable of secreting gas, but at a very low rate. This combination poses certain questions in regard to the behavior of the sardine and anchovy. For example, rapid addition of gas would be most useful during or immediately following descents to appreciable depths. Possibly these fish possess an unknown secreting mechanism, entirely different from that typical of physoclists. Alternatively, they do not descend to appreciable depths, or they do not have to maintain constant buoyancy in the process.

Whatever the hydrostatic functions of the bladder, relative pressure changes due to vertical movements would distort the prootic membrane. This membrane, if its primary function is oscillation, would require equal pressure on both sides for optimal performance. On this assumption, Evans' description of the precoelomic capillary extension of the bladder as a sort of Eustachian tube is reasonable. Hydrostatic-pressure changes transmitted through the lateral-sensory canal system and perilymphatic space would be balanced across the prootic membrane by gas-pressure changes transmitted through the precoelomic capillaries from the gas bladder. Of course, the length and, more particularly, the diameter of the gas-bladder capillaries would influence the rate at which equilibrium would be reestablished after a vertical movement.

In the sardine, there are no specializations for maintaining constant volume in the bladder during descents, but it is nevertheless possible that a constant relative pressure can be maintained. Air could not be added through the pneumatic duct

to maintain both volume and pressure against increasing external pressure unless the fish had gulped an excess of it at the surface before starting to descend, and such a behavior pattern is unlikely. If, however, the relative pressure is maintained at the expense of volume by contraction of the bladder wall, the fish would become heavier. This would maintain the prootic membrane in equilibrium, and the depths to which the fish could descend would be limited by the degree of negative buoyancy it could sustain dynamically.

During rapid ascents, where the situation is reversed, relative pressure could be kept reasonably constant by relaxation of the bladder muscularis and the escape of gas through the postanal opening of the bladder. The pneumatic duct also affords a passage for the escape of gas, but because of its muscular structure, and the fact that it opens into the digestive tract, this tube could not be as sensitive to relative pressure changes as is the postanal duct.

The presence of the septum, and what is perhaps a pumping mechanism in the gas bladder of the anchovy, suggests that this species, which also presumably maintains a pressure equilibrium across the prootic membrane, has a somewhat different pattern of behavior than does the sardine. If, for example, gas is pumped from the posterior to the anterior chamber of the bladder during descent, the vertical range over which the fish could maintain the prootic membrane in equilibrium would be extended. Conversely, pumping gas out of the anterior chamber during rapid ascent would facilitate the reduction of pressure at the prootic membrane. A considerable pressure could probably be tolerated in the posterior chamber until sufficient gas escapes through the pneumatic duct.

Experimental investigation of the functions of the gas bladder, its cranial vesicles, and the ear will yield considerable information on the behavior of clupeoid fishes. Either the sardine or the anchovy would suffice for determination of the kind of function performed by the ear–gas bladder mechanism, but it would be yet more informative to determine whether the two species show differences of degree. Because of structural differences in the bladders of the two species, the hydrostatic functions and the functional relation between the bladder and cranial gas vesicles should be investigated in both species.

SUMMARY

As one approach to understanding availability of the Pacific sardine, *Sardinops caerulea*, and the northern anchovy, *Engraulis mordax*, behavior studies of these species are planned. Since it is essential to know the anatomy of the animals before embarking on such work, the anatomy of both species is now being investigated. In this paper, the gas bladder and its relation to the inner ear are described.

1. The basic relation between the cranial extensions of the gas bladder and the inner ear is the same in the Pacific sardine and the northern anchovy as in other clupeoids that have been investigated.

2. The form of the gas bladder differs in the sardine and anchovy. In the sardine, the pneumatic duct arises from the posterior end of the stomach blindsac, the bladder is single-chambered and has a postanal opening to the exterior. In the anchovy, the pneumatic duct arises from the cardiac stomach, the bladder is two-chambered and does not have a postanal opening to the exterior.

3. In the sardine, the upper and lower halves of the pneumatic duct differ in structure. The muscularis and the arrangement of septa in the lower half constitute a valve. The muscularis is thinner and septa are lacking in the upper half of the duct. In the anchovy, the duct is shorter and uniform in structure. The muscularis and arrangement of septa constitute a valve, as in the sardine.

4. The pneumatic duct in both species probably permits gas but not solids to pass from the stomach to the gas bladder. It would also permit the passage of gas from the gas bladder to the stomach.

5. The wall of the gas bladder in the sardine is composed of mucosa and tunica externa. The mucosa consists of simple squamous epithelium and muscularis mucosa. The muscularis mucosa is well developed; its fibers are transversely alined anterior to the insertion of the pneumatic duct and longitudinally alined posterior to the insertion. The tunica externa is a layer of loose fibroelastic connective tissue that varies in thickness.

6. The wall of the gas bladder in the anchovy is composed of mucosa and tunica externa. The mucosa consists of simple squamous epithelium

and muscularis mucosa. The muscularis mucosa is transverse in both the anterior and posterior chambers. Between the two chambers there is a thick, transverse septum followed by a thick-walled, constricted region. There is a well-developed, longitudinal muscle layer surrounding the thick, transverse muscle layer in this region.

7. The muscularis of the bladder wall in these two species probably maintains the form of the bladder during changes in volume at constant relative pressure. The septum and middle, constricted portion in the anchovy strongly suggests a peristaltic pumping mechanism capable of moving gas from one chamber to the other.

8. The genital and urinary openings are controlled by a massive sphincter, but the postanal opening of the gas bladder in the sardine is not controlled by a sphincter mechanism.

9. The posterior caecum of the gas bladder of the anchovy consists of a muscular tube which terminates in a very small, membranous chamber that is filled by epithelial lamellae. Its function cannot be ascertained without further study.

10. The function of the ear–gas bladder mechanism is discussed for both species. The hydrostatic functions of the gas bladder and the functional relation between the gas bladder proper and the ear-gas bladder mechanism are considered for each species.

11. Because of differences in cranial construction, the anchovy would probably be more amenable to surgery involving the inner ear-gas bladder mechanism than would the sardine.

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